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CONTENTS

Pyrazinamide-Isoniazid in Tuberculosis. Walsh McDermott, Louise Ormond, Carl Muschenheim, Kurt Deuschle, Robert M. McCune, Jr., and Ralph Tompsett 319
Observations on the Combined Use of Pyrazinamide (Aldinamide) and Isoniazid in the Treatment of Pulmonary Tuberculosis. A Clinical Study. Maurice Campagna, Arthur A. Calix, and George Hauser 334
The Relationship of Stress, Adrenocortical Function, and Tuberculosis. Edmund R. Clarke, Jr., Daniel W. Zahn, and Thomas H. Holmes 351
The Treatment of Tuberculous Meningitis. A Study of Forty-Six Adult Patients. Martin J. Fitzpatrick 370
Response to BCG Vaccination as an Index of Familial Susceptibility to Tuberculosis. Sven Nissen Meyer and Carroll E. Palmer 383
Air Embolism Complicating Pneumoperitoneum. A Review. William A. Dasher, J. P. Myles Black, William Weiss, and Emil Bogen 396
Studies on the Tuberculoinhibitory Properties of Ascorbic Acid Derivatives and Their Possible Role in Inhibition of Tubercle Bacilli by Urine. Quentin Myrvik, Russell S. Weiser, Burton Houglum, and Leslie R. Berger 406
Experimental Embolic Pulmonary Tuberculosis in Mice. Edmund Mayer, Edith R. Jackson, Eleanor S. Whiteside, and Clara Alverson 419
Case Reports:
Observations on Pyrazinamide (Aldinamide) in Pulmonary Tuberculosis. Samuel Phillips, John C. Larkin, Jr., Willard L. Litzenberger, Glenn E. Horton, and James S. Hadmsohn 443
Fatal Allergic Reaction to Para-Aminosalicylic Acid. Wilbur J. Steininger, Morris D. Kloppenstein, and C. Eugene Woodruff 451
Near-Fatal Shock from PAS Followed by Guillain-Barrés Syndrome. K. G. Bulley 455
The Goitrogenic Effect of Para-Aminosalicylic Acid During the Therapy of Pulmonary Tuberculosis. Report of Eleven Cases. Geoffrey L. Brinkman and E. Osborne Coates, Jr. 458
Notes:
Study of the Virulence of Isoniazid-Resistant Tubercle Bacilli in Guinea Pigs and Mice. A Preliminary Report. W. C. Morse *et al.* 464
The Antagonism of the Tuberculostatic Action of Isoniazid by Hemin. Myron W. Fisher 469
Isoniazid-Resistance and Catalase Activity of Tubercle Bacilli. A Preliminary Report. Gardner Middlebrook 471
Correspondence: Filterable Forms of *Mycobacterium Tuberculosis*. Georges H. Werner 473
Books 475
American Trudeau Society: Notes 477
Abstracts 57

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PYRAZINAMIDE-ISONIAZID IN TUBERCULOSIS[1,2,3]

WALSH McDERMOTT, LOUISE ORMOND,[4] CARL MUSCHENHEIM, KURT DEUSCHLE,[5] ROBERT M. McCUNE, Jr.,[6] AND RALPH TOMPSETT

With the technical assistance of Kurt Stern

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INTRODUCTION

During the past sixteen months, the present writers have been investigating the antituberculous effects produced by pyrazinamide and isoniazid, administered together, in a series of related studies in animals and in man. The animal studies with pyrazinamide-isoniazid represent part of a much larger investigation of antituberculous chemotherapy which will be presented elsewhere in detail (1). Certain of the findings in animals which are particularly relevant to pyrazinamide-isoniazid are briefly summarized in the present report, which consists of a detailed presentation of the results of the clinical investigation.

On the basis of the observations to be presented, it has been concluded that pyrazinamide-isoniazid exerts a high degree of antituberculous action *in vivo* of a sort superior and qualitatively different from that of current antituberculous drugs used either singly or together. It has been further concluded, however, that the high incidence of hepatitis in man during the administration of pyrazinamide-isoniazid makes their use together in present dosage an inadvisable procedure in the treatment of tuberculosis.

[1] From the New York Hospital-Cornell Medical Center and the Navajo Medical Service, Fort Defiance, Arizona.

[2] This study was supported in part by grants from the Navajo Tribal Council; the Committee on Medical Research of the American Trudeau Society, medical section of the National Tuberculosis Association; the Division of Research Grants and Fellowships, National Institutes of Health, Public Health Service; grants from the Lederle Laboratories Division, American Cyanamid Company, Pearl River, New York; and Chas. Pfizer & Co., Inc., Brooklyn, New York.

[3] The pyrazinamide (Aldinamide, Lederle) and the isoniazid (Nydrazid, Squibb) used in this study were generously supplied by the manufacturers: Lederle Laboratories Division, American Cyanamid Company, and E. R. Squibb and Sons, Division of Mathieson Chemical Corporation, New York, respectively.

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General Background

Pyrazinamide

The initial demonstrat on that pyrazinamide exerted a high degree of antituberculous action in man was made by Yeager, Munroe, and Dessau (2). In careful and cautiously interpreted studies, these investigators observed uniform and rapid defervescence and a reasonably satisfactory incidence of roentgenographic improvement among a group of 43 patients with pulmonary tuberculosis. They further observed, however, that in some patients the defervescence and other indices of improvement were not maintained despite the continuation of the pyrazinamide. Such relapses during therapy appeared as early as the eighteenth treatment day but, more commonly, after five or six weeks of treatment. Moreover, a few post-treatment relapses occurred and were uninfluenced by the readministration of pyrazinamide.

These initial observations by Yeager and his associates have been amply confirmed by other investigators (3, 3*a*), including the present group.

Subsequent studies of pyrazinamide in a number of laboratories revealed that it was not possible to demonstrate significant activity of the drug against *M. tuberculosis in vitro*. Moeover, in unpublished observations in this laboratory, it was not possible to demonstrate in human subjects that blood withdrawn after ingestion of pyrazinamide exerted antituberculous activity *in vitro*.

Pyrazinamide-Isoniazid

As a consequence of the demonstration that pyrazinamide had impressive, although frequently short-lived, antituberculous activity in humans, the use of the drug concurrently with other drugs has been studied by a number of investigators. Schwartz and Moyer, at Oteen, North Carolina, have given pyrazinamide along with streptomycin, para-aminosalicylic acid (PAS), or isoniazid, respectively, to three groups of tuberculous patients. Preliminary observations on these three groups of patients have been reported at a Chemotherapy Conference of the Veterans Administration (3). The results at two to four months in the first 16 patients who received isoniazid with the pyrazinamide were sufficiently promising for these investigators to recommend further trial of this regimen. Another investigation of pyrazinamide-isoniazid in humans has been conducted by Campagna, Calix, and Hauser[2] in New Orleans.

PYRAZINAMIDE-ISONIAZID IN EXPERIMENTAL ANIMALS

The McCune-Tompsett studies of pyrazinamide-isoniazid in mice, which were conducted in this laboratory, were made with the microbial enumeration technique (1). This technique, originally developed by Fenner, Martin, and Pierce (4), permits remarkably precise enumeration of the total po,ulation of *M. tuberculosis* (expressed as viable units) within an organ or tissue. By use of sufficiently large groups of animals and appropriate sampling proce lures, it is possible to determine the influence of introduced variables on the total po,ulation of *M. tub rculosis* within an organ during periods of several or more months in length.

Only one aspect of the McCune-Tompsett studies with this technique is directly germane to the present discussion and is briefly summarized as follows:

A difference exists between the lung and the spleen of the mouse with respect to the influence of various drugs on populations of tubercle bacilli within these organs.

[2] Reported in this issue of the REVIEW.

In the lung, a tissue in which the untreated infection is steadily progressive, the population of tubercle bacilli neither rises nor falls during ninety to one-hundred twenty days of streptomycin therapy. With isoniazid the microbial population falls sharply and in many instances drops below the limits of detection.

In the spleen, a tissue in which the untreated infection progresses at first and then stabilizes, the administration of isoniazid results in an immediate drop in the microbial census, which then stabilizes and remains constant for the experimental period despite continued administration of the drug. With streptomycin the microbial population in the spleen actually increases during the first ten days and then returns to slightly below the original census, where it remains despite continued therapy thereafter.

These phenomena are noted when drug and infection are started on the same day. When chemotherapy is started three weeks after infection, the results are qualitatively similar although the fall in population, which occurs only with isoniazid, is appreciably less marked.

The uniform persistence of a constant census of tubercle bacilli in the spleen, despite continued chemotherapy, is a phenomenon which was subjected to especially detailed studies.

Two sets of observations are worthy of particular emphasis. First, the populations of tubercle bacilli which persist at a constant level in the spleen despite continued chemotherapy are not drug-resistant in the customary sense. They are easily inhibited by appropriate concentrations of the corresponding drugs when tested by conventional methods *in vitro*. Second, in an extensive series of experiments, it was not possible to modify in any way the phenomenon of the persistence of tubercle bacilli in the spleen by administration of large doses of isoniazid, streptomycin, pyrazinamide, streptomycin-isoniazid, streptomycin-PAS, isoniazid-PAS, or isoniazid-streptomycin-PAS.

In contrast, when pyrazinamide and isoniazid were administered together, the population of tubercle bacilli in the spleen behaved in a strikingly different fashion. During the first two weeks of therapy (drugs and infection started the same day) the fall in the population of tubercle bacilli in the spleens of the pyrazinamide-isoniazid animals was identical with the fall in the spleens of the animals which received pyrazinamide alone. The population fall in the isoniazid animals was more rapid and greater than in the other two groups. From this point on, the populations in the pyrazinamide and in the isoniazid animals stabilized and remained constant throughout the remainder of the three-month treatment period. With the pyrazinamide-isoniazid animals, however, the census of the population of tubercle bacilli did not stabilize but, instead, the fall continued at a rapid rate until bacilli could no longer be detected in the spleens of any of the animals.

In short, pyrazinamide and isoniazid together produced an antimicrobial action which was qualitatively different from that produced by any of the standard antituberculous drugs administered either singly or together. Indeed, the effects produced by pyrazinamide-isoniazid were of a much more eradicative type than those produced by the other drugs and, conceivably, may have represented complete sterilization of the spleens of the treated animals. The last-named point is being subjected to detailed investigation at present and it should be possible to establish with certainty whether or not complete sterilization of the mouse spleen can be produced in this manner. In other experiments, an attempt is being made to find out the length of time pyrazinamide must be administered to produce the pyrazinamide-isoniazid effect. Stained smears of the tissues have revealed no acid-fast bacilli when tubercle bacilli were not detected by culture of the aliquots of splenic emulsion.

The results of the population studies in the lungs during pyrazinamide-isoniazid therapy were identical with those in the spleen.

Further experiments in mice with established pulmonary lesions, i.e., twenty-one days after infection, in which the pyrazinamide-isoniazid regimen was com-

pared with the effects of the two drugs individually, have given results which were identical with the initial experiments. A series of studies *in vitro*, designed to attempt to demonstrate the unique property of the pyrazinamide-isoniazid regimen, have thus far been totally unsuccessful.

* * *

The present clinical investigation of pyrazinamide-isoniazid was conducted on the medical services of the Navajo Medical Center, Fort Defiance, Arizona, and the New York Hospital-Cornell Medical Center. A total of 81 adolescents and adults with pulmonary tuberculosis were treated with the concurrent administration of both drugs followed by isoniazid alone.

MATERIALS AND METHODS

Type of tuberculous lesions: Roentgenographic abnormalities with the characteristics of tuberculous lesions were present in the chest films of all patients. In all but 9 of the 81 patients, the pulmonary lesions at the start of therapy were designated *far advanced* or *moderately advanced* by the 1952 classification of the National Tuberculosis Association.

Previous chemotherapy: Only 3 of the 81 patients had received any antituberculous chemotherapy, and in no instance had such therapy been received during the year prior to the start of pyrazinamide-isoniazid.

Measurement of roentgenographic changes: Serial roentgenograms were interpreted periodically and changes were recorded, using the customary categories of *marked improvement*, *moderate improvement*, *slight improvement*, *no change*, and *worse*.

For the purposes of the present report these categories have been reduced to three: *substantial improvement* (marked or moderate); *no significant improvement* (slight improvement or no change); and *worse*.

Microbiologic studies: M. tuberculosis was cultured before treatment from sputum or gastric washings of all but 8 of the 61 patients who received pyrazinamide-isoniazid therapy for at least three months. In the 8 patients with sterile cultures, microscopic examination of stained smears of sputum revealed acid-fast bacilli at the time antimicrobial therapy was started.

After the start of treatment, specimens for culture were obtained from all patients at approximately four-week intervals. In addition, during two separated weeks in June, 1953, and the first week in November, 1953, sputum and gastric washings from all patients who had received both drugs were stained for acid-fast bacilli and cultured for *M. tuberculosis*.

Isoniazid-susceptibility in vitro: Determinations of isoniazid-susceptibility *in vitro* were conducted in liquid medium (Tween-albumin) after primary isolation on solid medium (oleic-albumin–penicillin or Petragnani).

General management: All of the 81 patients were hospital inpatients who were kept on bed rest with some individual modifications, such as the allowance of bathroom privileges.

Tests of hepatic function: Determinations of serum bilirubin, sulfobromphthalein sodium retention, and thymol turbidity tests were used in the evaluation of hepatic function.

Pyrazinamide-isoniazid regimen: Pyrazinamide was administered in a total daily dosage of 50 mg. per kg. of body weight and isoniazid in a total daily dosage of 5 mg. per kg. Both drugs were given orally in equally divided doses twice daily.

In the first 16 patients treated, the two drugs were given together for approximately six months with isoniazid administered alone thereafter. As evidence of hepatic toxicity accumulated, the pyrazinamide regimen was shortened to ninety days. When further hepatic toxicity led to the decision to stop pyrazinamide altogether, 18 patients had received the two drugs together for various intervals from two to ten weeks. Two additional patients died from tuberculosis on the fifth and eleventh days of therapy.

In all but 2 patients, isoniazid was continued after completion of the pyrazinamide-isoniazid therapy. The two exceptions represented 2 of the patients who had developed hepatitis during the sixth month of therapy.

OBSERVATIONS

Sixty-one patients received pyrazinamide-isoniazid for at least three months, with isoniazid alone thereafter.

Only two measurable components of the course of infection during treatment will be considered: roentgenographic changes; and the status of infectiousness, as determined by culture of sputum and gastric washings.

Roentgenographic Course

Six months: Six months after the start of therapy, the roentgenographic course of the 55 patients for whom data were available was designated *substantial improvement*[8] in 42 patients; no *significant improvement* in 13 patients; and *worse* in no patients.

Nine months: Nine months after the start of pyrazinamide-isoniazid therapy, the roentgenographic categorizations in the 25 patients for whom data were available were: *substantial improvement*[9] in 19 patients, *no significant change* in 6; and *worse* in no patients.

Cavity closure: Cavity closure (all cavities in each patient), as determined roentgenographically, occurred in 23 of the 36 patients with clearly demonstrable cavities at the start of therapy. The cavity closures occurred during the first six months after the start of pyrazinamide-isoniazid with the exception of two patients whose cavity closures occurred eight months after the start of therapy.

Status of Infectiousness

The status with respect to infectiousness, as determined by smear and culture of sputum and gastric washings, was analyzed for all of the 61 patients who had completed at least three months of pyrazinamide-isoniazid therapy. Six of the 61 patients had left the hospital before the three-month bacteriologic studies could be obtained. In every one of these 6 patients, however, post-treatment bacteriologic studies made at some time before the three-month point had failed to disclose tubercle bacilli. An additional 2 patients whose pre- and post-treatment sputum was consistently negative for tubercle bacilli on both microscopic examination and culture were likewise excluded. There remained, therefore, 53 patients on whom three- and six-month bacteriologic observations were available. Specimens for culture were obtained at approximate four-week intervals from all patients. In addition, during two separated weeks in June, 1953, and the first week in November, 1953, sputum and gastric washings from all patients who had received both drugs were stained for acid-fast bacilli and cultured for *M. tuberculosis*.

[8] Marked improvement, 20 patients; moderate improvement, 22 patients.

[9] Marked improvement, 11 patients; moderate improvement, 8 patients.

The results of the cultural studies are presented in terms of the number of months following the start of pyrazinamide-isoniazid therapy and are as follows: *M. tuberculosis* was isolated from 4 of 53 patients at three months; from the same 4 of 53 patients at six months; and from 2 of 31 patients at nine months.

The 2 patients designated as infectious at nine months represented 2 of the 4 who were discharging bacilli at six months and at three months. Both of these patients have become noninfectious following excisional surgery.

Thus, more than 90 per cent of the group of 53 patients was noninfectious within three months of the start of pyrazinamide-isoniazid therapy. The same group was also noninfectious at the six-month observation point, and its 29 members who have been observed for nine months were likewise noninfectious at that time.

The only indication of a renewal of the infectious state which has been observed has been the finding at twelve months of acid-fast bacilli in the sputum of a woman with a roentgenographically demonstrable cavity. Cultures of sputum obtained at the same time failed to reveal tubercle bacilli.

Although 47 of the 53 patients were noninfectious at three months and thereafter, the actually demonstrated incidence of reversal of infectiousness[10] was slightly lower. The reason for this is the inclusion of the 8 patients from whom *M. tuberculosis* was not isolated on the single pretreatment culture or thereafter. Justification for the inclusion of these 8 patients rests on the fact that acid-fast bacilli were readily demonstrated on microscopic examination of the pretreatment sputum obtained from all of the 8 patients; and the roentgenographic findings and course (including cavity in 4) were characteristic of pulmonary tuberculosis. When these 8 patients were excluded from analysis, the actually demonstrated incidence of reversal of infectiousness was 41 of 45 patients, or 90 per cent.

Presence of cavity in relation to status of infectiousness: To a certain extent, the reversal of infectiousness during chemotherapy in a group of patients with pulmonary tuberculosis is a reflection of the extent of regression of the individual lesions. Among the 53 pyrazinamide-isoniazid patients, all but 4 of whom were noninfectious at six months, a roentgenographically demonstrable cavity or cavities was present at that same time in 14 patients.

Microscopic examination of stained smears of sputum: Appropriately stained smears were examined for acid-fast bacilli at the two June and one November, 1953, cultural examinations mentioned above. The results of the two methods showed good general agreement. In a few instances, acid-fast bacilli could be detected at the time of the first culture negative for *M. tuberculosis* but disappeared thereafter.

Isoniazid-susceptibility in vitro: Data on the isoniazid-susceptibility *in vitro* of the patient-strains of tubercle bacilli during therapy are fragmentary, for

[10] The term *reversal of infectiousness* is used merely to designate the situation when, despite repeated attempts, tubercle bacilli can no longer be cultured from sputum or gastric washings of patients who were previously discharging such bacilli. It is not intended to imply that the state of noninfectiousness is necessarily permanent.

only 4 patients continued to discharge bacilli after the first four to eight weeks of pyrazinamide-isoniazid therapy. Three of these patient-strains, isolated at three months, were drug susceptible (inhibited by 0.13γ of isoniazid per ml.) and one patient-strain was resistant (not inhibited by 25γ of isoniazid per ml.). Determinations at six months (3 patient-strains) and nine months (2 patient-strains) revealed no subsequent change in the status of the patient-strains tested.

Hepatitis

The only definite manifestation of drug toxicity noted was the appearance of unequivocal evidence of impairment of hepatic function in 6 patients. The abnormalities in function were accompanied by icterus in 4 of the patients and, in 3, the hepatitis was severe.

One of the severe reactions occurred in a 45-year-old woman, who died of rapidly progressive hepatitis eight days after the first evidence of drug intolerance appeared on the fifty-fifth treatment day. Another patient with severe hepatitis has made a complete recovery with normal values for hepatic function but only after experiencing a severe illness with marked icterus and ascites. The third patient's illness was only slightly less severe. She has made a complete symptomatic recovery, but abnormal values for hepatic function persist.

Examination of hepatic tissue obtained by biopsy in the second patient and at autopsy in the fatal case revealed the characteristic findings of a severe chemical hepatitis.

The hepatitis was first detected in the fatal case on the fifty-fifth treatment day and in an alcoholic patient with suspected hepatic cirrhosis on the ninetieth treatment day. The other 4 cases were the first to occur, and all appeared during the sixth month of therapy. Consequently, it was at first believed that shortening the pyrazinamide administration to ninety days might provide a satisfactory margin of safety. The subsequent appearance on the fifty-fifth treatment day of the reaction which rapidly proved fatal resulted in the discontinuance of the pyrazinamide altogether.

The six instances of hepatitis were equally distributed between the Arizona and the New York Services. As 66 patients received pyrazinamide-isoniazid for fifty-five or more days, the incidence of hepatitis in the present series was approximately 10 per cent. The remainder of the patients showed no evidence of impairment of hepatic function at any time, and the 5 patients with nonfatal hepatitis have completely recovered except for the persistence of abnormal values for hepatic function in the one patient.

DISCUSSION

Treatment of 55 patients with pulmonary tuberculosis on a regimen of pyrazinamide-isoniazid for three months or longer and on isoniazid alone thereafter resulted in sustained reversal of infectiousness in 90 per cent, substantial roentgenographic improvement in 75 per cent, and cavity closure in 65 per cent of those with cavitary lesions. These results were all attained within the first six months of therapy except for later cavity closure in 2 patients. No

patient showed evidence of progression of disease on roentgenography. In the portion of the group for whom nine months' observations are available (approximately one half), the results have remained unchanged.

Three patients died: 2, of tuberculosis within the first eleven days of therapy; and one, of hepatitis on the fifty-fifth day of therapy. These 3 patients are not included in the 55 patients cited above, all of whom completed at least three months of therapy. Five instances of nonfatal hepatitis occurred among the 61[11] patients between the third and the sixth months of pyrazinamide-isoniazid therapy.

Evaluation of the antimicrobial activity of a drug in pulmonary tuberculosis is a very difficult procedure for two principal reasons. In the first place, roentgenographic densities may be quite similar despite variations in the character and potentialities of the pathologic processes involved. Second, it has been shown by actual trial that assignment of pulmonary lesions viewed on serial roentgenograms into even such crude categories as "better," "worse," or "no change" is associated with a fairly wide margin of error (5). Nevertheless, despite the relative crudity of the method, roentgenographic studies have quite properly been the principal reliance in evaluations of antituberculous drugs. The chief reasons for this practice are the facts that the method is uniformly applicable in all cases of pulmonary tuberculosis and the other manifestations of this disease are even less susceptible to regular measurement.

In the present study with pyrazinamide-isoniazid, the results by roentgenographic evaluation were superior to the results observed in a series of patients with pulmonary tuberculosis who were treated with isoniazid alone by the same investigators (6). A 75 per cent incidence of substantial roentgenographic improvement and no instances of disease progression were observed at six and nine months on pyrazinamide-isoniazid in the present study in contrast to an incidence of substantial improvement of 55 per cent with a 7 per cent incidence of disease progression among the patients who received only isoniazid (6). Moreover, this 75 per cent incidence of substantial roentgenographic improvement with no disease progressions is apparently superior to the 50 to 59 per cent incidence of substantial improvement and 5 to 7 per cent incidence of disease progression reported in the U.S.P.H.S. cooperative studies of isoniazid, streptomycin-PAS, and isoniazid-streptomycin (7).

The question immediately arises, however, whether differences of this order are meaningful or whether they merely represent chance differences which could fall within the limits of the method.

Early in the present investigation, this question arose with particular force when it became apparent that the incidence of substantial roentgenographic improvement was running higher than it was on isoniazid alone. It was true that the incidence of substantial roentgenographic improvement was apparently higher in the pyrazinamide-isoniazid study. It was also true, however, that the

[11] This number includes all who received three months or more of pyrazinamide-isoniazid irrespective of whether roentgenographic or microbiologic follow-up observations are available.

patients in both studies were receiving an admittedly powerful antituberculous drug, isoniazid. Moreover, approximately one half of the patients in the isoniazid series had previously received various amounts of streptomycin-PAS although, in almost all cases, no such treatment had been received during the three months prior to isoniazid therapy.

It appeared that any regimen which included isoniazid in appropriate dosage resulted in 50 to 60 per cent substantial roentgenographic improvement. Hence the suspicion arose that, because of the histopathologic difference in apparently comparable lesions, the roentgenographic method might not be ideally suited for the conclusive demonstration of significant differences between two regimens, *both of which contained isoniazid*.

Accordingly, it was decided to attempt to resolve the problem by recourse to information which was being obtained by two other methods: microbiologic studies of sputum and gastric washings and the population-enumeration studies in mice.

The results of the microbiologic studies of sputum and gastric washings were clear and definite. Ninety days after the start of therapy, only 4 of 53 patients were continuing to discharge bacilli. Once this noninfectious status was attained, it persisted throughout the total observation period of six to nine months. (It should be noted that all but 2 patients continued to receive isoniazid after completion of pyrazinamide-isoniazid therapy.) In most instances, no culture obtained after the first month of treatment was positive for *M. tuberculosis*.

The last-named finding was unexpected and caught the investigators unawares, thus accounting for the fact that only a single pretreatment culture had been obtained on any of the patients. In 8 of the group, this single pretreatment culture failed to grow *M. tuberculosis* although, in all cases, the pretreatment sputum contained acid-fast bacilli on microscopic examination. Even when these 8 cases are excluded from consideration, however, the established incidence of the reversal of infectiousness is 90 per cent. This 90 per cent incidence of reversal of infectiousness on pyrazinamide-isoniazid may be compared with a comparably estimated incidence of 50 per cent at six and at nine months in the previous study with isoniazid alone (6).

A comparison of the microbiologic results of the present study with those of the two U.S.P.H.S. cooperative studies (6, 7) can be made when the present results are presented in terms of the number of patients from whom tubercle bacilli could be cultured during treatment. In the present study, at three, six, and nine months after the start of chemotherapy, tubercle bacilli could be cultured from only 7 per cent of the patients. In approximately the same interval in the first Public Health Service study (7), cultures positive for tubercle bacilli were obtained in 20 to 30 per cent of the patients despite the fact that streptomycin-PAS and isoniazid-streptomycin were used as well as isoniazid alone. In a subsequent U.S.P.H.S. study (8), various combinations of isoniazid with PAS and streptomycin were investigated. The regimens included the administration of all of these drugs together and two regimens (isoniazid-streptomycin, isoniazid-PAS) in which an isoniazid dosage of 10 mg. per kg. was used. At sixteen

weeks, in this later study, tubercle bacilli could be cultured from 9 to 21 per cent of the patients, depending upon the particular regimen employed.

It is to be anticipated that some of the patients in the present study who were discharging no tubercle bacilli at three, six, and nine months after the start of therapy, will again become infectious if their pulmonary lesions persist unhealed. Thus far, however, the only suggestion of such a happening has been the case of the patient with a persistent cavity at twelve months, whose sputum contained acid-fast bacilli on one microscopic examination at that time. Sputum cultures obtained from this patient have been repeatedly negative for *M. tuberculosis*.

The apparent superiority of pyrazinamide-isoniazid over isoniazid alone observed by the roentgenographic method has thus been associated with a definite superiority of pyrazinamide-isoniazid in terms of the sustained reversal of infectiousness. The McCune-Tompsett studies of populations of tubercle bacilli in mice provided the third body of evidence in favor of the unique superiority of pyrazinamide-isoniazid.

In essence, their animal studies showed that it was possible to produce effects with pyrazinamide-isoniazid in mice which could not be produced by pyrazinamide alone or by isoniazid, streptomycin, or PAS, administered singly, in various pairs, or all three together. These pyrazinamide-isoniazid effects consisted of the rapid reduction of a large population of tubercle bacilli in the spleen to the point at which no tubercle bacilli could be detected by multiple cultures. With all of the other combinations tested, after an initial fall, the population census in the spleen stabilized and remained constant throughout treatment periods of ninety to one hundred and twenty days. The substantially greater antituberculous activity of pyrazinamide-isoniazid was also demonstrable in the microbial population studies in the lung.

The extent to which observations on drug–parasite–host relationships in mice pertain to what goes on in man is not at all clearly defined. Indeed, if the population enumeration studies in mice constituted the only evidence bearing on the respective activities of pyrazinamide-isoniazid and of isoniazid alone, a close resemblance of the results of the mouse studies to the situation in the tuberculous patient might be seriously questioned. There are three sets of circumstances, however, which indicate that the behavior of the populations of tubercle bacilli in the mice treated with pyrazinamide-isoniazid does have significant relevance to the situation in man.

The three sets of circumstances are: First, the evaluation of other antituberculous drugs (isoniazid, streptomycin, PAS, and a thiosemicarbazone) by population enumerations in mice has, without exception, yielded results in close agreement with the respective activities of these drugs as determined in man (1). Second, the pyrazinamide phenomenon of substantial antituberculous activity for five or six weeks with "escape" thereafter occurs in identical fashion in man and in the mouse. Third, the mouse data with pyrazinamide-isoniazid are not isolated phenomena but, instead, yield results in striking agreement with the results of the microbiologic and roentgenographic observations in man.

It is believed that, considered individually, the roentgenographic results might

conceivably reflect merely chance variations within the roentgenographic method and the mouse data might represent drug action peculiar to that animal species. When the two sets of observations are considered together, however, and the sputum studies are likewise considered, the results by all three methods fit together into an impressive case for the superiority of pyrazinamide-isoniazid over the other antituberculous drugs used singly or concurrently.

The possibility that the pyrazinamide-isoniazid phenomenon represents antimicrobial action of an eradicative rather than a suppressive type is worthy of comment. Two examples exist of a reasonably uniform eradicative type of drug action in human infections, namely, the sulfonamide treatment of meningococcus carriers, and the use together of pentaquine and quinine in the treatment of vivax malaria (8*a*). Even if the studies currently in progress should reveal that pyrazinamide-isoniazid is completely eradicative for *M. tuberculosis* in mice, however, it would not necessarily follow that the situation would be the same in man. Examples exist in other infections, notably syphilis, in which penicillin is uniformly eradicative for *T. pallidum* in one animal species, the rabbit, but is not uniformly so in man. Indeed, in the present study, tubercle bacilli could be cultured from the sputum of 2 patients nine months after the start of therapy and from the tissues of the patient who died of hepatitis on the fifty-fifth treatment day.

It is clear, therefore, that pyrazinamide-isoniazid does not exert a uniformly eradicative action against *M. tuberculosis* in man. Nevertheless, the results observed in the present study in humans, considered with the pronounced sterilizing or eradicative type of action in mice, suggest strongly that the pyrazinamide-isoniazid action in humans represents a considerable more "destructive type" of action against *M. tuberculosis* than is the case with the other antituberculous chemotherapies.

Despite the apparent superiority of pyrazinamide-isoniazid to the presently available forms of antituberculous chemotherapy, its potentialities for widespread use appear to be sharply restricted by considerations of drug toxicity. All drug toxicity is a relative matter, and it is impossible to define the toxicity of a chemotherapeutic regimen out of the context of the situation in which the regimen is to be used. In addition to providing powerful antimicrobial therapy for the hospitalized tuberculous patient, the pyrazinamide-isoniazid regimen has two attributes of great potential value for the treatment of tuberculosis in the home. The two attributes are: freedom from any gastrointestinal irritation, which might tend to discourage the continued consumption of the medication as prescribed; and the ability to cause a rapid and sustained disappearance of tubercle bacilli from the sputum in a very high percentage of patients with pulmonary tuberculosis. Hence, in addition to providing powerful chemotherapy for any patient, one of the great spheres of usefulness of the regimen, if drug toxicity were not a problem, would be in the home treatment of infectious patients in the many areas throughout the world where there is no possibility of attaining adequate hospital facilities for tuberculosis in the foreseeable future.

In order to be acceptable for such widespread and relatively unsupervised use

in tuberculosis, a drug must be not only nonirritating to the digestive system but its more serious forms of toxicity should be detectable by patient or family before irreversible harm is done. It is precisely in this latter respect that the present pyrazinamide-isoniazid regimen has been such a lamentable failure. To be sure, if tests of hepatic function, which are relatively complicated, could be obtained at frequent intervals, the use of pyrazinamide-isoniazid in the dosage of the present study might be a relatively safe procedure. It should be strongly emphasized, on the basis of the present experience in the present study, however, that it would be necessary to obtain such tests of hepatic function at two- or three-week intervals to ensure proper safeguards for the patient. Moreover, in the fatal case of hepatitis, the initial complaints were deceptively trivial. On the third or fourth day, by the time the presence of hepatitis could first be established, the illness had become explosive, and terminated fatally within less than a week thereafter.

An obviously alluring possibility is that the high degree of antituberculous effectiveness of pyrazinamide-isoniazid observed with the present dosage would also be exerted with a lower dosage which at the same time would be reasonably nontoxic. These two questions are under detailed investigation by the present investigators. Certain aspects of the question, notably the size of the total daily dose of the pyrazinamide necessary to produce the pyrazinamide-isoniazid effect, can probably be well defined only in man. The necessary duration of the pyrazinamide administration in the joint therapy can be studied in both animals and man. Continued observation of the 18 patients of the present study who received only two to ten weeks of the two drugs together may provide some information on this question. However, this prospect that the length of pyrazinamide administration might be shortened without significant loss in the effectiveness of the regimen does not appear too encouraging. For, from preliminary results of certain of the animal studies, it appears that the two drugs must be administered together for at least six to eight weeks (with isoniazid alone thereafter) in order to duplicate the effects previously described for the ninety-day regimen. Hence, it appears at present that the greatest hope for reducing the drug toxicity of the regimen lies in making a considerable reduction in the size of the total daily dose of pyrazinamide.

The six instances of hepatitis were equally distributed between the Arizona and the New York Services. The respective roles of the pyrazinamide itself and the isoniazid-pyrazinamide regimen in producing the hepatitis cannot be determined, for the present investigators have used pyrazinamide alone in only a few patients. In the original study of pyrazinamide (2) by Yeager and his associates, however, hepatitis with icterus was noted in 2 of 43 patients. Hepatitis from isoniazid is rare, if indeed it occurs at all, on the 3 to 5 mg. per kg. daily dose. Nevertheless, it is known that considerably larger doses of isoniazid can produce hepatitis in dogs (9). Hence, it is conceivable that customarily nontoxic amounts of isoniazid might become toxic to a liver simultaneously damaged by pyrazinamide.

It is obviously of the greatest importance to answer these questions about

pyrazinamide-isoniazid hepatitis and to conduct investigations aimed at its prevention or avoidance. For, unless this can be accomplished, the benefits of this powerful and unique type of antituberculous chemotherapy will necessarily be limited in their application.

SUMMARY

The antituberculous activity of pyrazinamide-isoniazid administered together has been studied in separate but related investigations in man and in experimentally infected mice. The results of the clinical investigation are described in the present report, in which the animal studies are only briefly reviewed.

Treatment of 55 patients with pulmonary tuberculosis on a regimen of pyrazinamide-isoniazid for three months or longer and isoniazid alone thereafter resulted in sustained reversal of infectiousness in 90 per cent; substantial roentgenographic improvement in 75 per cent; and closure of all cavities in 65 per cent of those with cavitary lesions. These results were attained within the first six months of therapy (except for later cavity closure in 2 patients). No patient showed progression of disease on roentgenography. In the portion of the group for whom nine-month observations are available (approximately one half), the results have remained unchanged.

Three patients, not included in the above group, died: 2 of tuberculosis within the first eleven days of therapy and one of drug-induced hepatitis on the fifty-fifth day of therapy. Five instances of nonfatal hepatitis were also observed.

From the evidence obtained, it is concluded that pyrazinamide-isoniazid exerts antituberculous activity *in vivo* of a sort superior to that of the other current antituberculous drugs used either singly or together. Moreover, the animal studies indicate that the antimicrobial action of pyrazinamide-isoniazid is not only quantitatively superior but is qualitatively unique among antituberculous chemotherapies.

It is further concluded, however, that the high incidence of hepatitis in man during pyrazinamide-isoniazid administration makes the use of this regimen in present dosage inadvisable as a treatment for tuberculosis.

SUMARIO

La Piracinamide-Isoniacida en la Tuberculosis

La actividad antituberculosa de la piracinamida-isoniacida administradas conjuntamente fué estudiada en investigaciones separadas, pero relacionadas, en el hombre y en ratones infectados experimentalmente. Los resultados de la investigación clínica son descritos en este trabajo, en el cual se repasan, pero sucintamente, los estudios en animales.

El tratamiento de 55 tuberculosos pulmonares sometidos a un régimen de piracinamida-isoniacida durante tres meses o más y a isoniacida sola después, dió por resultado un viraje sostenido de la infecciosidad en 90 por ciento, importante mejoría radiográfica en más de 70 por ciento, y cierre de todas las cavernas en 65 por ciento de los que tenían lesiones cavitarias. Esos resultados se alcanzaron durante los primeros seis meses de terapéutica (aparte de clausura tardía de cavernas en 2 enfermos). Ningún enfermo reveló roentgenográficamente agravación de la dolencia. En la porción del grupo (aproximadamente la mitad) para la cual se cuenta con nueve meses de observación, los resultados han permanecido inalterados.

Tres enfermos, que no figuran en el grupo anterior, fallecieron: dos de tuberculosis dentro de los primeros once días de terapéutico y uno de hepatitis medicamentosa a los 55 días de terapéutica. Observáronse además 5 casos de hepatitis no letal.

A la luz de los datos obtenidos, dedúcese que la piracinamida-isoniacida despliega *in vivo* actividad antituberculosa de un género superior al de las demás drogas antituberculosas usadas hoy día ya aislada o conjuntamente. Además, los estudios realizados en animales indican que la acción antimicrobiana de la piracinamida-isoniacida no tan sólo es cuantitativamente superior sino cualitativamente única entre los agentes quimioterapéuticos antituberculosos.

No obstante, dedúcese además que la elevada incidencia de hepatitis en los seres humanos durante la administración de piracinamida-isoniacida convierte en imprudente el empleo de ese régimen a las dosis actuales como tratamiento para la tuberculosis.

RESUME

Pyrazinamide-Isoniazide dans la tuberculose

L'activité antituberculeuse de l'association de la pyrazinamide et de l'isoniazide a été étudiée dans des investigations séparées, mais connexes, chez l'homme et aussi chez la souris tuberculisée. Les résultats de l'étude clinique sont décrits dans cet article; l'expérimentation sur l'animal est succintement analysée.

Le traitement de 55 sujets atteints de tuberculose pulmonaire par l'association thérapeutique pyrazinamide-isoniazide, pendant trois mois ou plus, et par l'isoniazide seule ensuite, a eu pour résultat constant la suppression de la contagiosité dans 90 pour 100 des cas; une amélioration substantielle des radiogrammes dans plus de 70 pour 100 des cas et l'oblitération de toutes les cavernes dans 65 pour 100 des cas présentant des lésions cavitaires. Ces résultats ont été atteints dans les six premiers mois de la thérapie (sauf chez deux malades où l'oblitération des cavernes a été plus tardive). Aucun malade n'a présenté de signes radiologiques d'evolution de la maladie. Dans la fraction du groupe qui a été observé pendant neuf mois (environ la moitié) les résultats sont demeurés inchangés.

Trois malades non compris dans le groupe sont morts: deux de tuberculose au cours des 11 premiers jours du traitement et l'autre des suites d'une hépatite toxique au bout de 55 jours de cette thérapeutique. Cinq cas d'hépatite non mortelle ont été également observés.

Les preuves obtenues, permettent de conclure que l'association pyrazinamide-isoniazide exerce une action anti-tuberculeuse *in vivo* présentant une supériorité particulière sur celle des autres médicaments anti-tuberculeux courants utilisés seuls ou en association. De plus, les recherches effectuées sur l'animal indiquent que l'action antimicrobienne de l'association pyrazinamide-isoniazide n'est pas seulement quantitativement supérieure, mais aussi qualitativement unique parmi les médications anti-tuberculeuses.

Les auteurs concluent d'autre part que, chez l'homme, l'incidence élevée de l'hépatite au cours de l'administration de la pyrazinamide associée à l'isoniazide, fait déconseiller l'emploi de cette médication, aux doses actuelles, comme traitement de la tuberculose.

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OBSERVATIONS ON THE COMBINED USE OF PYRAZINAMIDE (ALDINAMIDE) AND ISONIAZID IN THE TREATMENT OF PULMONARY TUBERCULOSIS[1,2]

A Clinical Study

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INTRODUCTION

Although previous reports of the use of pyrazinamide (Aldinamide) in the chemotherapy of pulmonary tuberculosis have been disappointing (1) chiefly because of the rapid emergence of drug-resistant bacilli, it was believed that the usefulness of this agent might be enhanced if it were combined with isoniazid.

Pyrazinamide was synthetized at the Lederle Laboratories by Kushner and his associates (2). It was subsequently shown by Dessau and co-workers (3) and by Malone and his associates (4), who used mice and guinea pigs respectively, that pyrazinamide had a moderate but definite antituberculous effect against these animals; this activity was superior to that of para-aminosalicylic acid (PAS) but less than that of streptomycin. Yeager and his associates then used pyrazinamide alone in the treatment of 43 cases of far advanced pulmonary tuberculosis (1). Most of the patients had received streptomycin and PAS in the past and harbored organisms which were resistant to streptomycin. The pyrazinamide therapy of these patients was characterized by encouraging signs, such as rapid reduction of fever and amelioration of the cough, with a decrease in expectoration. The end results were disappointing, however, because relapse was extremely common and the emergence of drug-resistant strains of tubercle bacilli occurred rapidly.

The present investigation began in June, 1952, and included a group of 21 patients with far advanced pulmonary tuberculosis, all of whom had a sputum positive for *M. tuberculosis* and most of whom had been treated unsuccessfully with streptomycin and PAS and various forms of collapse therapy. This report is based on an eighteen-month period of observation.

PLAN OF INVESTIGATION

Patients from the Tuberculosis Clinic of the New Orleans Health Department were used in the present investigation. A suitable group of patients with active far advanced disease who had not responded favorably to streptomycin and PAS (most cases) and various forms of collapse therapy was selected. All of these patients had sputum positive for tubercle bacilli by microscopic examination and cultures, and all had poor prognoses.

Pyrazinamide was administered in capsules three times a day. Early in this study a dosage of four 350 mg. capsules daily was used. After two months the dosage was changed to

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[1] From the Division of Tuberculosis Control, New Orleans Health Department; Section of Chronic Disease and Tuberculosis, Louisiana State Department of Health; and Division of Laboratories, Louisiana State Department of Health.

[2] Presented before the Southern Trudeau Society at the meeting of the Southern Tuberculosis Conference at New Orleans on October 1, 1953.

3 gm. daily in divided doses. Isoniazid was administered concurrently in the usual daily dose of 4 mg. per kg. of body weight.

Early in the study, it was believed that it might be advantageous to perform drug-susceptibility studies, using a combination of isoniazid and pyrazinamide. This was not done, however, because of various technical difficulties which were foreseen. Determination of drug resistance *in vitro* in the case of pyrazinamide is difficult and the results are unreliable (5, 6). Because of this, it was decided to test for susceptibility to isoniazid alone.

Before treatment was started, a careful history with a summary of past treatment was taken and a physical examination was performed. The following laboratory data were obtained in addition to the sputum smears and cultures: complete blood count, liver function studies (cephalin flocculation, bromsulfalein retention), and urinalysis.

The problem of close follow-up of these patients was solved by soliciting the cooperation of field public health nurses. A special form with check boxes was devised so that the nurses could point out any untoward symptoms which might arise during the course of chemotherapy, together with the usual signs and symptoms of tuberculosis. This enabled us to determine quickly which patients might be developing signs of toxicity to these agents. Special laboratory studies could then be performed without loss of time to determine whether these symptoms were serious enough to warrant discontinuing the drugs.

RESULTS

Drug Toxicity

Examination of the patients and a study of the special forms showing signs and symptoms of toxicity indicated that certain specific symptoms occurred during the administration of these drugs. These symptoms are listed in the order of their frequency.

Joint pains: Ten patients complained of joint pains. The joints involved were the following: shoulder (4 cases), knee and ankle joints (3 cases), ankle joint (2 cases), and the hip joint (one case). Redness and swelling of the joints was not noted in any case; the complaint was always subjective. The pain in these cases was never severe enough to warrant discontinuation of the medication and seldom lasted longer than two weeks.

Headaches: Two female patients complained of moderately severe frontal headaches which were associated with the menses. These headaches had occurred previous to treatment with these drugs but they were now more severe.

Jaundice: Mild jaundice appeared briefly for approximately one week in 3 of these patients. The liver function tests were not seriously altered in these cases. The medication was not discontinued since the jaundice disappeared and no definite alterations in the liver function tests occurred (table 1).

Difficulty in urination: Three male patients complained of some difficulty during micturition. This symptom was easily relieved by local application of heat.

Antituberculous Activity

Bacteriologic findings: During the first thirty days of therapy, sputum "conversion" did not occur in any patient. In 12 patients, the sputum became negative for *M. tuberculosis* by culture during the second month. In 2 other patients, the sputum likewise became negative during the third month and, in 5 additional patients, the sputum became negative for tubercle bacilli during the fourth month of the study. These patients had sputum negative for tubercle bacilli for

TABLE 1

EFFECT OF COMBINED USE OF PYRAZINAMIDE AND ISONIAZID IN TREATMENT OF TWENTY-ONE PATIENTS WITH PULMONARY TUBERCULOSIS

PATIENT	BROMSULFALEIN RETENTION 5 MG./KG. (MAYO MOD)								CEPHALIN FLOCCULATION							
	Initial Liver Function		After 6 Months		After 12 Months		After 18 Months		Initial		After 6 Months		After 12 Months		After 18 Months	
	min	*per cent*	*min*	*per cent*	*min*	*per cent*	*min*	*per cent*	*hours*		*hours*		*hours*		*hours*	
M. Bua	15 45	26 8	15 45	16 2	15 45	30 2	15 45	36 4	24 48	Negative Negative	24 48	Negative Negative	24 48	Negative Negative	24 48	Negative Negative
M. Hutton*	15 45	12 Trace	15 45	36 4	15 45	28 2	15 45	40 4	24 48	Negative Negative	24 48	Negative Negative	24 48	Negative Negative	24 48	Negative Negative
M. McGrath	15 45		15 45	22 4	15 45	28 2	15 45	28 4	24 48	Negative 1+	24 48	Negative Negative	24 48	Negative Negative	24 48	Negative Negative
F. Viguerie	15 45	28 1	15 45	28 2	15 45	20 2	15 45	32 2	24 48	Negative Negative	24 48	Negative 1+	24 48	1+ 1+	24 48	Negative Negative
J. Varnado	15 45	12 4	15 45	23 1	15 45	26 0	15 45	36 4	24 48	Negative Negative	24 48	Negative Negative	24 48	1+ 1+	24 48	2+ 2+
R. Boland	15 45	2.8 9	15 45	16 5	15 45	32 4	15 45	16 2	24 48	Negative Negative	24 48	Negative Negative	24 48	1+ 1+	24 48	2+ 2+
P. Yost*	15 45	16 4	15 45	38 14	15 45	44 8	15 45	28 10	24 48	Negative Negative	24 48	Negative Negative	24 48	1+ 1+	24 48	Negative Negative
K. Ferguson	15 45	[illegible]	15 45	18 2	15 45	20 0	15 45	12 2	24 48	Negative Negative	24 48	Negative Negative	24 48	Negative Negative	24 48	Negative Negative
B. Daniels	15 45	6 0	15 45	27 1	15 45	36 4	15 45	32 4	24 48	Negative Negative	24 48	Negative 1+	24 48	Negative Negative	24 48	Negative Negative
J. Wright	15 45	36 20	15 45	29 16	15 45	56 20	15 45	48 20	24 48	2+ 2+	24 48	Negative Negative	24 48	2+ 3+	24 48	2+ 2+
L. Rivera	15 45	18 2	15 45	88 54	15 48	48 16	15 45	36 2	24 48	Negative Negative	24 48	2+ 2+	24 48	Negative 1+	24 48	2+ 2+

M. Keys	15 45	36 8	15 45	32 6	15 45	40 4	15 45	28 2	24 48	Negative 1+	24 48	Negative Negative	24 48	Negative Negative	24 48	Negative Negative
R. Williams	15 45	12 Trace	15 45	21 6	15 45	40 4	15 45	32 2	24 48	Negative Negaitve	24 48	Negative Negative	24 48	Negative Negative	24 48	1+ 1+
R. McClendon	15 45	24 4	15 45	23 4	15 45	32 4	15 45	24 2	24 48	Negative Negative	24 48	2+ 2+	24 48	1+ 1+	24 48	2+ 2+
R. Johnson	15 45	5 0	15 45	22 4	15 45	20 0	15 45	32 5	24 48	Negative Negative	24 48	1+ 1+	24 48	Negative Negative	24 48	2+ 2+
A. Smith	15 45	18 10	15 45	No test 6	15 45	40 0	15 45	No test 8	24 48	Negative Negative	24 48	2+ 2+	24 48	Negative Negative	24 48	Trace 1+
E. Mason	15 45	14 2	15 45	30 8	15 45	40 4	15 45	32 8	24 48	Negative Negative	24 48	Negative Negative	24 48	Negative Negative	24 48	2+ 2+
S. Dominick*	15 45	14 4	15 45	18 4	15 45	24 2	15 45	30 8	24 48	Negative Negative	24 48	2+ 2+	24 48	Negative Negative	24 48	1+ 1+
M. Johnson	15 45	20 Trace	15 45	76 41	15 45	20 2	15 45	24 4	24 48	Negative Negative	24 48	2+ 3+	24 48	Negative Negative	24 48	2+ 2+
G Carter	15 45	36 3.2	15 45	42 12	15 45	32 4	15 45	40 6	24 48	Negative 1+	24 48	1+ 3+	24 48	Negative Negative	24 48	2+ 2+
H. Williams†	15 45	14 Trace	15 45	36 8	15 45	20 0	†	†	24 48	Negative Negative	24 48	Negative 2+	24 48	Negative Negative		† †

* Patients who showed jaundice.
† Moved out of city following thoracoplasty; Unable to locate.

TABLE 2

EFFECT OF COMBINED USE OF PYRAZINAMIDE AND ISONIAZID IN TREATMENT OF TWENTY-ONE PATIENTS WITH PULMONARY TUBERCULOSIS

Erythrocyte Count and Hemoglobin Concentrations

INITIAL BLOOD COUNT AND HEMOGLOBIN		AFTER 6 MONTHS OF CHEMOTHERAPY		AFTER 12 MONTHS OF CHEMOTHERAPY		AFTER 18 MONTHS OF CHEMOTHERAPY	
Erythrocyte Count (Cells per Cu. Mm.)	Hemoglobin* (Gm.)	Erythrocyte Count (Cells per Cu. Mm.)	Hemoglobin* (Gm.)	Erythrocyte Count (Cells per Cu. Mm.)	Hemoglobin* (Gm.)	Erythrocyte Count (Cells per Cu. Mm.)	Hemoglobin* (Gm.)
White Female							
4,270,000	11.0	5,110,000	13.4	4,320,000	14.0	4,940,000	13.5
4,250,000	12.0	5,540,000	12.8	4,170,000	12.8	4,080,000	12.0
4,040,000	10.0	4,760,000	13.4	4,400,000	14.0	4,600,000	14.0
4,080,000	11.5	5,200,000	12.8	4,400,000	13.0	4,600,000	15.0
Negro Female							
4,040,000	12.5	5,370,000	13.4	4,400,000	13.8	4,480,000	14.5
4,030,000	12.0	4,830,000	16.4	4,100,000	13.0	4,290,000	14.5
4,400,000	14.5	5,430,000	16.4	4,510,000	14.0	4,570,000	14.5
3,100,000	5.5	5,550,000	10.0	3,390,000	9.5	4,310,000	11.5
3,500,000	10.0	5,300,000	13.6	3,500,000	11.0	3,470,000	12.5
3,700,000	11.0	4,400,000	12.4	4,260,000	12.5	4,100,000	12.5
4,430,000	12.0	5,350,000	15.0	4,430,000	15.0	4,610,000	15.5
White Male							
4,560,000	14.5	6,350,000	18.0	5,230,000	16.0	5,760,000	17.5
4,560,000	14.5	5,490,000	16.6	5,030,000	15.5	5,250,000	15.5
4,000,000	14.5	5,070,000	14.4	4,510,000	13.5	4,800,000	13.5
4,550,000	14.4	5,020,000	16.0	4,750,000	15.0	4,880,000	14.0
Negro Male							
4,970,000	15.0	7,000,000	18.4	5,960,000	16.5	4,770,000	14.5
4,490,000	14.5	5,300,000	16.0	4,510,000	14.0	5,430,000	15.0
5,320,000	14.8	5,660,000	17.4	4,820,000	15.0	4,520,000	16.0
4,140,000	11.5	5,910,000	16.0	5,300,000	15.5	4,840,000	13.8
4,400,000	14.0	4,700,000	15.0	4,800,000	14.5	4,110,000	12.0
5,230,000	15.0	6,700,000	19.5	5,480,000	16.0	†	†

* Normal 14.5 gm. per 100 cc.

† Moved out of city following thoracoplasty; unable to locate.

the entire eighteen-month period of observation and continue to have such negative sputum at present. Two of the patients failed to show sputum "conversion" at any time. Up to this writing, the tubercle bacilli isolated from these 2 patients show resistance to isoniazid *in vitro*. All patients showed a disappearance or decided decrease in sputum volume.

Roentgenographic observations: Definite improvement in the roentgenographic

abnormalities could be demonstrated in the films of at least 11 of the patients at the end of the six-month period. The changes observed were more marked in those who showed soft exudative lesions of recent origin than in those who had not received previous antituberculous therapy. Although the roentgenographic changes in many of the patients exhibiting fibrotic and cavitary disease were not spectacular, several showed definite favorable change in the size of the cavitary lesions.

Hematologic observations: Probably the most spectacular improvement noted was in the hematologic findings six months after the initiation of therapy. In some cases a polycythemia was noted. There was a consistent increase in the total erythrocyte count and in the concentration of hemoglobin. This change was observed even in the patients who failed to show sputum "conversion." The average increase in the erythrocyte count in the white males was 1,065,000 cells per cu. mm.; the average increase in the Negro males was 1,100,000 cells per cu. mm.; the increase in the white females was slightly less, 992,500; and the increase in the Negro females averaged 990,000 cells per cu. mm. This increase in erythrocytes was accompanied by an average increase of 2.5 gm. in the hemoglobin concentration of the entire group. Subsequent blood counts taken at six-month intervals showed a leveling off of the initial high level, but still a substantial increase in most of the patients, and a consistent improvement in the hemoglobin concentrations which apparently is permanent (table 2).

Body weight: There was a gratifying increase in the average weight of all but 2 of the patients, the same 2 who failed to show sputum "conversion." The average weight gain in the entire group was 13 pounds. The maximum gain observed was 33 pounds, and the minimum gain was but 2 pounds (table 3).

The patients have been treated for eighteen months and maintained under close observation. Three (15 per cent) have attained a favorable stage, and surgical intervention is now feasible. Eight (40 per cent) have definitely improved

TABLE 3

EFFECT OF COMBINED USE OF PYRAZINAMIDE AND ISONIAZID IN TREATMENT OF TWENTY-ONE PATIENTS WITH PULMONARY TUBERCULOSIS

Weight Before and After Therapy

WHITE MALE		WHITE FEMALE		NEGRO MALE		NEGRO FEMALE	
Before	After	Before	After	Before	After	Before	After
Pounds		*Pounds*		*Pounds*		*Pounds*	
164	173½	87½	93	119	131	78	84½
143	151	86	119	120	132½	96½	109
105	112	120	134	177	185	85¾	146
116	116	74	70	138½	151½	133	159
				124	126	118	126
				166	195	104¼	140
						100	112¼
132	140	92	104	140	153	110	125

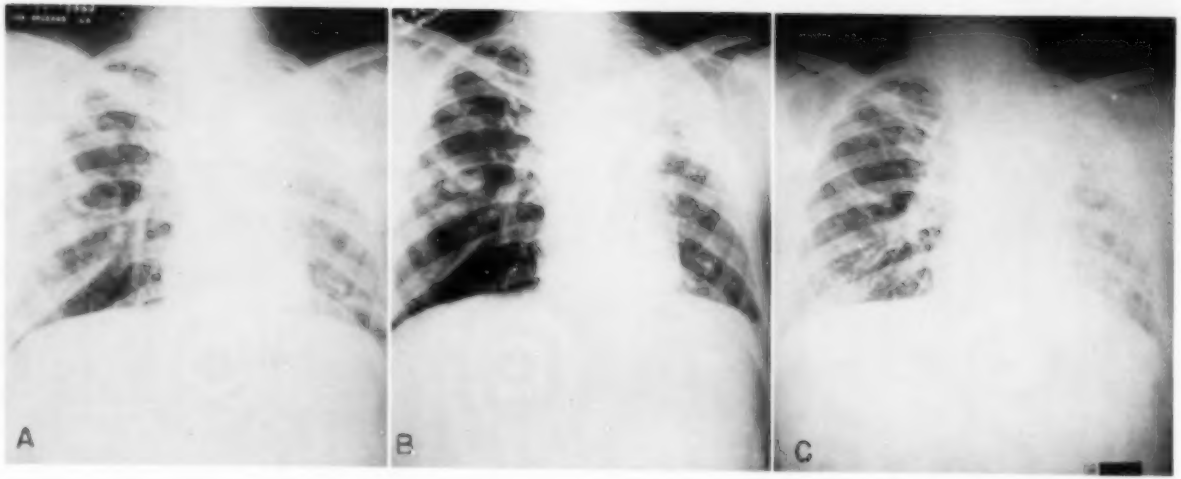

FIG. 1. P. W. (W/M/40)

A. Initial film prior to treatment with pyrazinamide and isoniazid. Inadequate previous therapy. Sputum positive for *M. tuberculosis;* weight, 164 pounds.

B. Six months after combined therapy. Weight, now 173½ pounds; sputum repeatedly negative for *M. tuberculosis* by culture. Note the diminution in size of cavity.

C. Eighteen months after chemotherapy, the cavity has completely closed. The sputum has remained negative for *M. tuberculosis*, and the patient now weighs 181 pounds. Evidence of closure of this cavity was apparent by roentgenogram at the twelfth month.

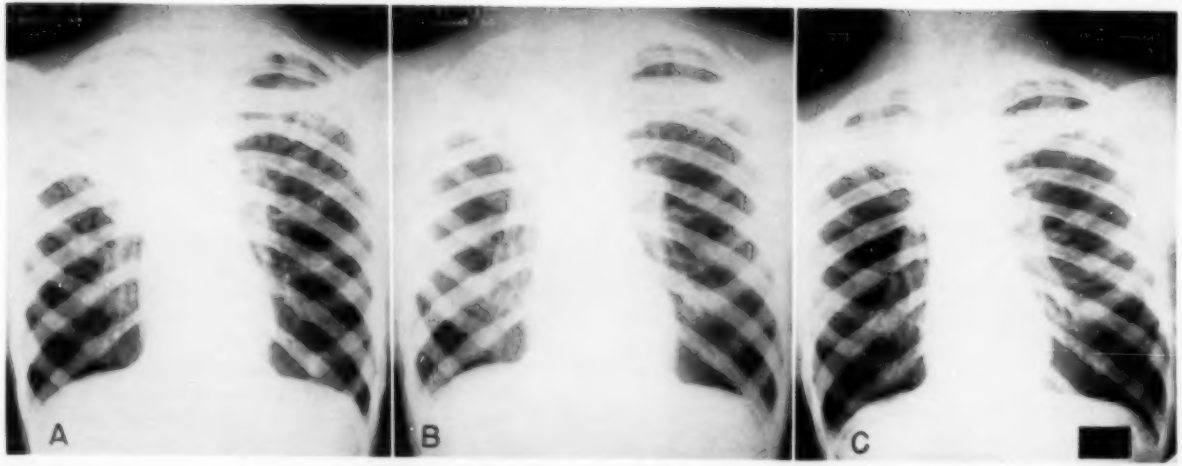

FIG. 2. G. C. (C/M/31)

A. Initial film prior to combined therapy. No previous treatment. In beginning of treatment, weight was 120 pounds. Note the cavity in the right upper lobe.

B. At the end of six months, the soft lesions in the left lung have disappeared.

C. The lesion in the right upper lobe has fibrosed completely, and there is now aeration throughout the previously diseased area. The patient remains well and the weight is now 130 pounds.

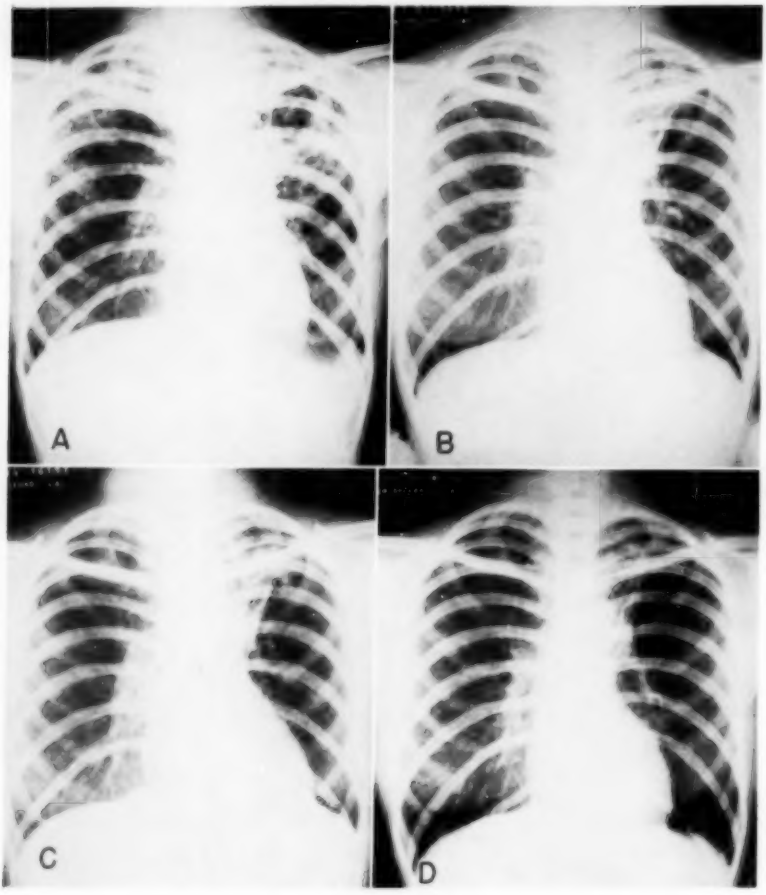

FIG. 3. S. D. (C/F/26)

A. Bilateral soft exudative lesions. Refused hospitalization. No previous treatment. Initial weight, 100 pounds.

B. and *C*. Six and eight months after treatment.

D. Eighteen months after initiation of therapy, the lesion appears to be completely fibrosed. The patient's sputum remains negative for *M. tuberculosis*, and her weight is now 112¼ pounds.

roentgenographically and clinically, and their tuberculosis can be classified as inactive. Others are not so fortunate in that they have thick-walled bilateral cavities and poor pulmonary function. Even in these patients, however, great symptomatic improvement has been noted, such as increase in weight, amelioration of anemia, a feeling of well-being, and, in all but 2 of this entire series, the sputum

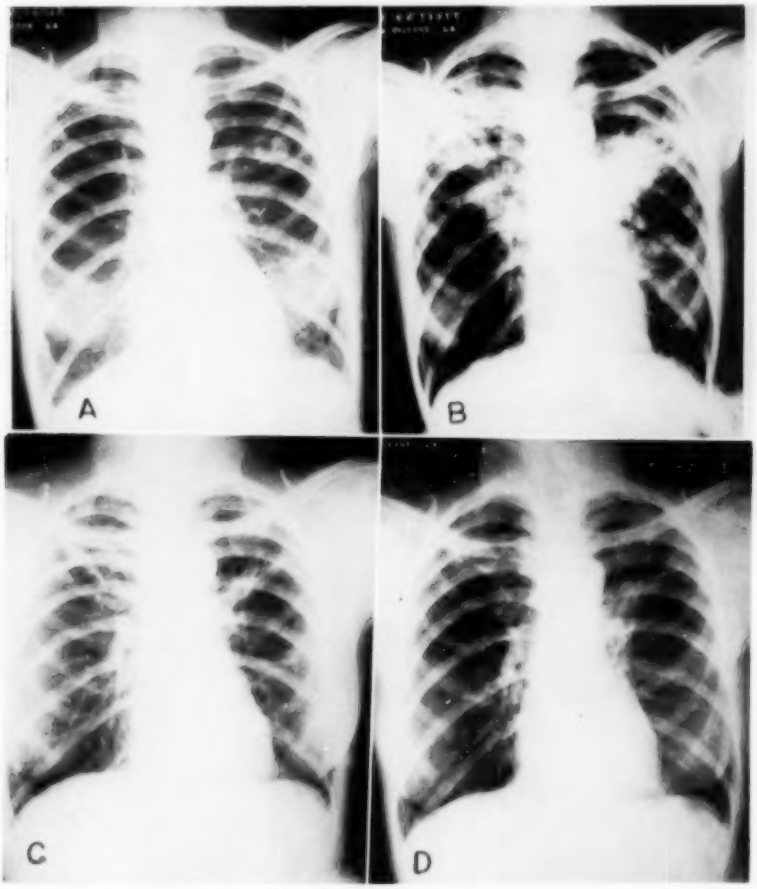

FIG. 4. M. J. (C/F/45)

A and *B*. *A* gives a history of a "cold" lasting twenty-three years. No previous roentgenograms. This film and *B* are four months apart. Treatment was started immediately after film *B*. Weight, 85¾ pounds.

C. Compared with *B*, there is a remarkable recession of lesions.

D. At the end of eighteen months of continuous combined therapy, the lesions appear quite fibrosed. The weight has gone up to 106 pounds. The increase in blood count demonstrated on the table has maintained a constant level.

has remained negative for tubercle bacilli by microscopic examination and culture. From a public health point of view this observation is highly significant.

DISCUSSION

The present writers are aware of the fact that others have had disappointing results with the use of pyrazinamide alone in the therapy of tuberculosis because

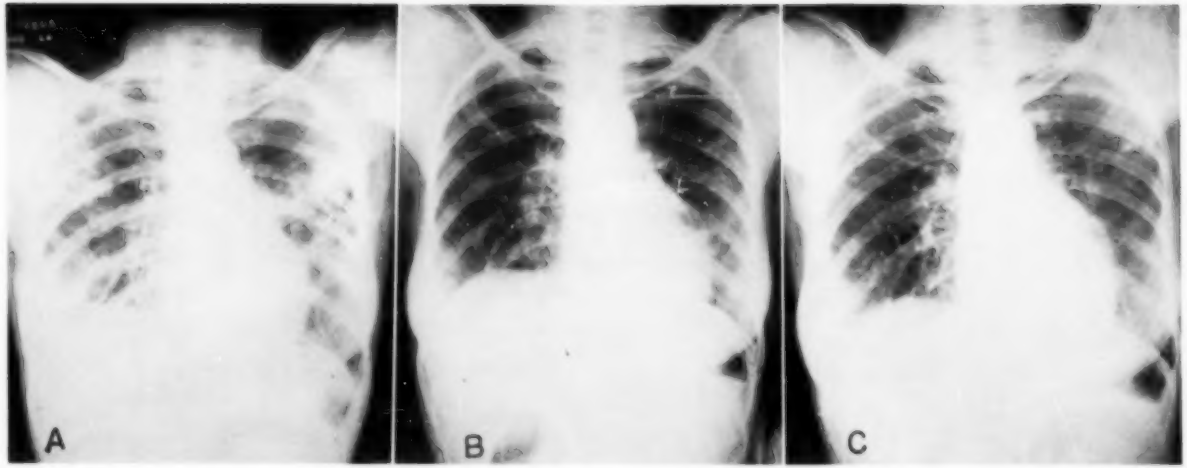

FIG. 5

A. Bilateral infiltration involving a considerable portion of both lungs with what appeared to be a well-defined cavity in the left lung field opposite the plane of the anterior arc of the third rib. This patient weighed 96½ pounds when treatment was started in July, 1952. The temperature was 101°F.

B. Right lung shows considerable clearing.

C. The lesions have cleared up considerably. There appears to be roentgenographic evidence, however, of possible persistence of the cavity in the left lung field. The sputum remains negative for *M. tuberculosis*, and the patient has gained 16½ pounds. Her blood count, which had increased from 3,700,000 to 4,400,000 cu. mm. in the first six months of treatment, has maintained this improvement, and at the end of eighteen months the count was 4,100,000 cu. mm. The hemoglobin originally was 11 gm. (Sahli). At the time of this film, it was 12.5 gm. (Sahli).

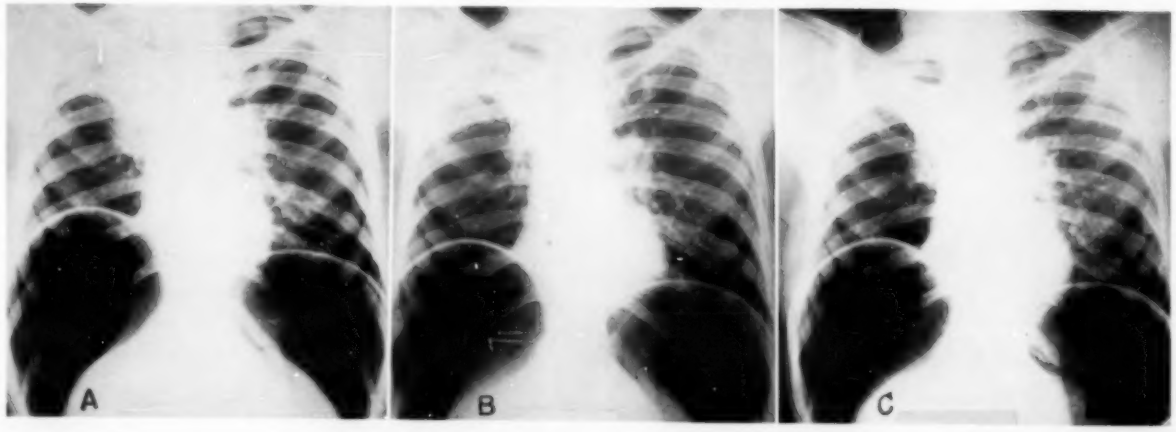

FIG. 6. E. M. (C/M/29)

A. This patient has received a course of streptomycin, para-aminosalicylic acid, and pneumoperitoneum before being put on the combined therapy. The sputum was positive for *M. tuberculosis* in spite of the treatment with streptomycin.

B. Twelve months after combined pyrazinamide and isoniazid therapy. The sputum has remained consistently negative for *M. tuberculosis* since the second month of treatment. The patient has gained 13 pounds and his blood count shows a polycythemia.

C. This film represents eighteen months of therapy. The blood count has leveled off, dropping gradually from 7,000,000 to 5,960,000 cu. mm. in August, 1953, and the present level is 4,770,000 cu. mm. On planigrams a cavity is demonstrated just behind the anterior arc of the right first rib. The patient has been offered surgery.

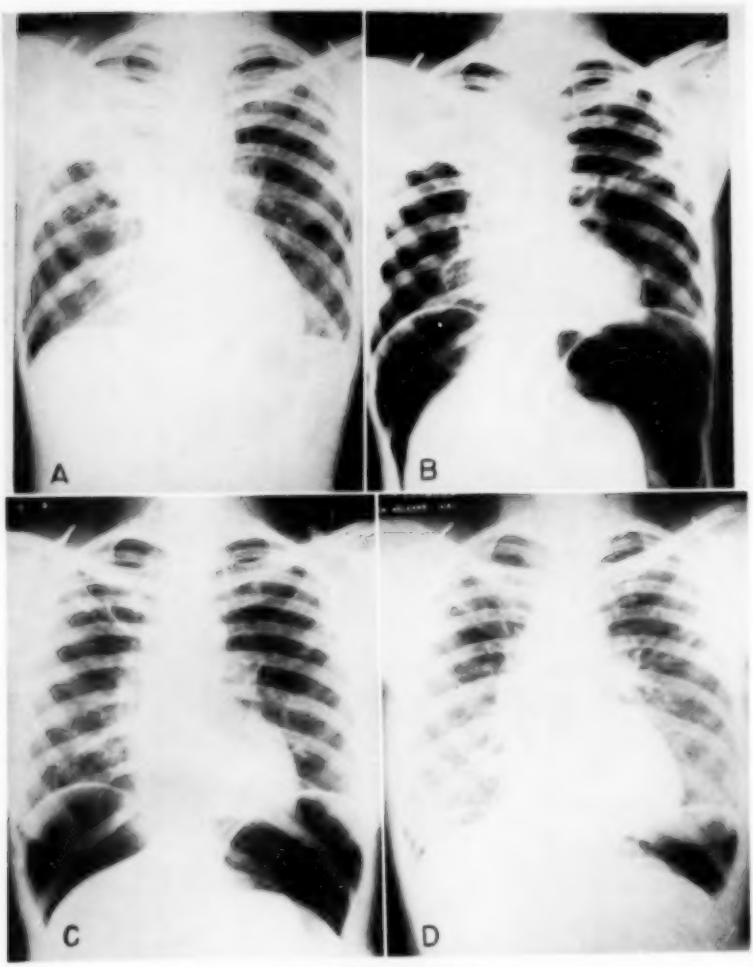

FIG. 7. B. D. (C/F/42)

A. This patient has had no previous treatment. Her weight at the beginning of treatment was 104½ pounds.

B. The lesion in the left lung has receded and the right lung pathology has contracted. Pneumoperitoneum was started in conjunction with chemotherapy.

C and *D*. At the end of one year and eighteen months, respectively, the residual lesions appear quite fibrotic. The sputum in this patient became negative for *M. tuberculosis* at the end of one month of treatment. The final weight is 143 pounds, an over-all gain of 39½ pounds.

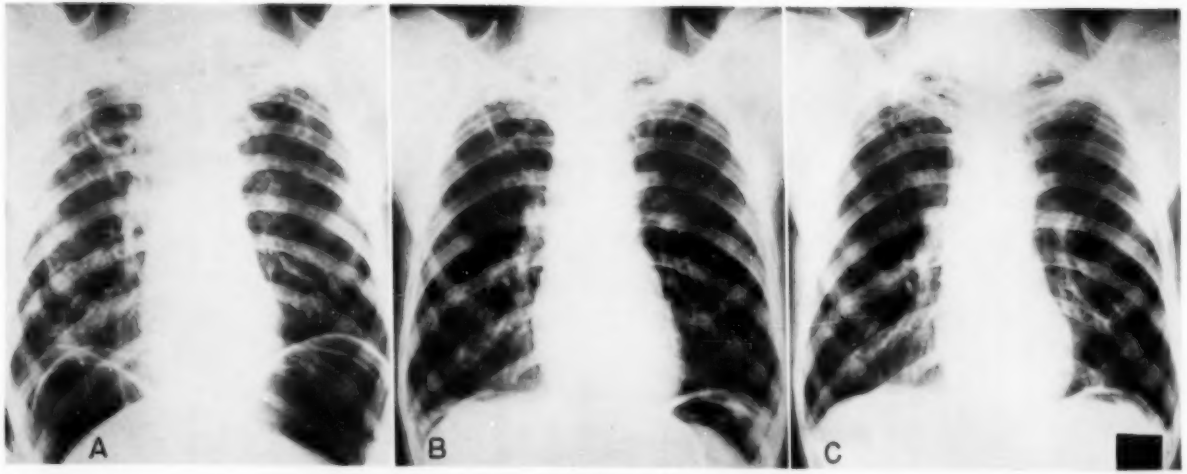

FIG. 8. J. W. (C/M/48)

A. This patient was in an institution for two years prior to inclusion in this series. He also received streptomycin, para-aminosalicylic acid, and pneumoperitoneum. His sputum was positive for *M. tuberculosis;* weight, 119 pounds.

B. After six months of therapy, note the receding cavity. The sputum has been negative for *M. tuberculosis* for the past three months.

C. The sputum continues to be negative for *M. tuberculosis* at the end of eighteen months. The patient's weight is now 139 pounds. This patient's hemoglobin has shown some decrease at the end of eighteen months.

of the rapid emergence of drug-resistant organisms and that certain workers have experienced difficulty in demonstrating an *in vitro* effect when using this drug alone (5, 6).

It has been well demonstrated that most of the known antituberculous compounds are more effective when used in combination (7–12). This is particularly true in the case of isoniazid, and physicians have been warned to avoid using this agent alone because of the rapid emergence of drug-resistant strains of organisms. Because of this it was believed that the effectiveness of pyrazinamide might be enhanced if administered concurrently with isoniazid. The results derived from the present study, in the writers' opinion, demonstrate that this combination is highly efficacious and superior to any that would be expected from the use of isoniazid alone or in combination with other known antituberculous agents (11, 13, 14).

Eleven of the patients studied showed an improvement in the status of their pulmonary tuberculosis, as demonstrated on serial roentgenograms. In other patients, whose original disease was of such nature (advanced fibrotic disease with thick-walled cavities) that no changes were expected, 3 (25 per cent), nevertheless, showed definite improvement in their chest roentgenograms; 8 patients (40 per cent) in the study can now be classified as having inactive tuberculosis (December, 1953).

The results obtained in the present study indicate that this combination of drugs lends itself readily to treatment of far advanced cases of pulmonary tuberculosis on an ambulatory basis.

It is planned to carry out a similar study on a group of minimal and moderately advanced active cases of pulmonary tuberculosis.

CONCLUSIONS

Concurrent administration of pyrazinamide and isoniazid is efficacious in the chemotherapy of pulmonary tuberculosis.

The toxic effects of this combination of drugs is negligible in most cases and not of serious consequence in the few cases in which such manifestations as jaundice and arthritis arose.

Persistent sputum "conversion" to date has occurred in all but 2 of the 21 patients treated.

Chest roentgenograms showed evidence of improvement in the majority of the patients treated even though little improvement was expected because of the nature of the original lesions.

Little improvement was seen in the roentgenograms of patients who had bilateral thick-walled cavities and poor pulmonary function.

These patients did show symptomatic improvement, however, and, as long as their sputum remains negative for tubercle bacilli, they are of no danger to the community. From a public health point of view, this observation is of great importance.

It is believed that this combination of drugs lends itself readily to treatment of far advanced tuberculosis on an ambulatory basis.

CONCLUSIONES

Observaciones Relativas al Uso Combinado de la Piracinamida (Aldinamida) y la Isoniacida en el Tratamiento de la Tuberculosis Pulmonar: Estudio Clínico

La administración concurrente de piracinamida y de isoniacida es eficaz en la quimioterapia de la tuberculosis pulmonar.

Los efectos tóxicos de esa combinación medicamentosa son menospreciables en la mayor parte de los casos y no fueron de mayor importancia en los pocos casos en los que se presentaron manifestaciones tales como ictericia y artritis.

Hasta la fecha ha habido "viraje" persistente del esputo en todos los 21 casos tratados, con excepción de 2.

Las radiografías torócicas revelaron signos de mejoría en la mayoría de los sujetos tratados, aunque era de esperar poca mejoría, dada la naturaleza de las lesiones primitivas.

Observóse poca mejoría en las radiografías de los enfermos que tenían cavernas bilaterales de paredes gruesas y mala función pulmonar.

No obstante, mostraron esos sujetos mejoría sintomática y, mientras permanezca su esputo negativo para bacilos tuberculosos, no revisten peligro para la colectividad. Desde el punto de vista sanitario, esta observación posee mucha importancia.

Parece que dicha combinación de drogas se presta fácilmente para el tratamiento de la tuberculosis muy avanzada a base ambulante.

CONCLUSIONS

Observations sur l'emploi combiné de la pyrazinamide (Aldinamide) et de l'isoniazide dans le traitement de la tuberculose pulmonaire: Une étude clinique

L'administration conjointe de la pyrazinamide et de l'isoniazide est efficace dans la chimiothérapie de la tuberculose pulmonaire.

Les effets toxiques de cette association médicamenteuse sont négligeables dans la plupart des cas et sans conséquences graves dans les rares cas où des manifestations telles que la jaunisse et l'arthrite se sont produites.

A ce jour, sur vingt-et-un malades traités, la "conversion" des crachats a été constamment observée chez tous les malades sauf deux.

Les radiographies du thorax montraient des signes d'amélioration chez la majorité des malades traités, même lorsqu'en raison de la nature des lésions existantes on ne s'attendait pas à observer d'amélioration.

Une légère amélioration a été observée dans les radiogrammes de malades affectés de cavernes bilatérales à parois épaisses et d'une fonction pulmonaire déficiente.

Ces malades montraient toutefois une amélioration symptomatique et, dans la mesure où leurs crachats demeuraient "négatifs", ils ne représentaient pas un danger pour la collectivité. Du point de vue hygiène publique, cette observation est de grande importance.

Les auteurs sont d'avis que cette association de médicaments se prête facilement au traitement ambulatoire des cas très avancés de la tuberculose.

Acknowledgment

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THE RELATIONSHIP OF STRESS, ADRENOCORTICAL FUNCTION, AND TUBERCULOSIS[1, 2, 3]

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INTRODUCTION

Many investigations have clearly demonstrated that adrenal hormonal imbalance exerts a profound effect upon resistance in tuberculosis (1–15). Other investigators have been able to demonstrate significant alterations in adrenocortical function in response to a variety of physical and emotional stresses (16–30). These observations suggest a possible physiologic mechanism by which stress may alter resistance to tuberculosis. The present writers have attempted to explore this possibility and to seek possible correlations between stress as reflected by emotional status, adrenocortical function, and the course of tuberculosis during treatment.

METHOD AND MATERIAL

Adrenocortical function was quantitatively estimated in the present study by determination of the urinary content of neutral 17-ketosteroids by a modification of the method of Drekter and associates (31, 32). The 17-ketosteroid determinations were performed on twenty-four-hour urine specimens collected shortly following admission and at intervals during the course of hospitalization. The results are expressed as milligrams per twenty-four hours.

One hundred and nine patients have been studied. For the most part, they were consecutive admissions to the hospital, although 4 patients with lengthy prior hospitalization were included. Ninety-two patients had pulmonary tuberculosis, and 17 had some other type of tuberculosis as the principal reason for treatment. One hundred and seven patients had tuberculosis in the active stage, and 2 were considered to have questionably active disease. All were receiving the usual combinations of hospital treatment, including chemotherapy during a major portion of the study period. It was not possible to establish a diagnosis of Addison's disease by either clinical or laboratory findings in any of the patients studied. Nevertheless, 4 patients gave a history of having had a prior diagnosis of Addison's disease, and 2 had received treatment for that disease at least four years prior to admission to the present study.

The course of tuberculosis was determined from changes in the visible lesions on serial chest roentgenograms and in bacteriologic or other laboratory data. Observations of attitude and behavior were made by the physician in immediate charge of the patient and, when necessary, were explored further in psychiatric consultation.

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[2] Presented before the Medical Session, as part of the session on *Tuberculosis and Related Subjects*, at the annual meeting of the National Tuberculosis Association, Los Angeles, California, on May 20, 1953.

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RESULTS

17-Ketosteroid Excretion Versus Age and Sex

The mean 17-ketosteroid excretion observed in the present series was 8.93 plus-minus 4.78 mg. per twenty-four hours for males and 8.61 plus-minus 2.65 mg. per twenty-four hours for females. This finding indicates a significant alteration of adrenocortical function. The similarity of mean excretion for both sexes is interesting in view of the fact that 17-ketosteroid excretion in males is generally higher than in females by approximately one-third, presumably due to androgenic substances (or their metabolites) arising from testicular tissue. However, Hamburger (33), Robinson (34), Kenigsberg and co-workers (35), and Sprechler (36) have shown that 17-ketosteroid excretion varies with age. As a large proportion of the patients in the present study were in the older age groups, it seemed advisable to consider the mean excretion in relationship to age. The mean excretions for males and females by age groups are presented in table 1. These findings, when compared to the mean excretion expected in nontuberculous subjects (35), indicate that tuberculous patients have reduced 17-ketosteroid excretion even when the factor of age is considered.

17-Ketosteroid Excretion Versus Character and Extent of Disease

In order to attach any significance to 17-ketosteroid excretion in a particular patient, it is necessary to compare the observed 17-ketosteroid excretion with

TABLE 1

MEAN 17-KETOSTEROID EXCRETION BY SEX AND AGE

AGE RANGE	FEMALES			MALES		
	Number	Observed mg. per 24 hours	Expected* mg. per 24 hours	Number	Observed mg. per 24 hours	Expected* mg. per 24 hours
						3.8
10–19	1	11.2		3	8.1 ± 5.4	
20–29	10	9.4 ± 2.7		11	11.5 ± 2.7	18.0
30–39	5	7.5 ± 1.5		13	10.2 ± 4.8	
40–49	5	9.0 ± 0.6	9.3	22	9.8 ± 4.2	15.0
50–59	4	7.0 ± 2.4		19	7.6 ± 2.8	
60–69	—	—		14	6.2 ± 2.8	9.0
70–	—	—		2	5.8 ± 1.3	
Total	25	8.6 ± 2.7	9.3 ± 2.0*	84	8.9 ± 4.8	16.0*

* Based upon data by Kenigsberg and associates (35).

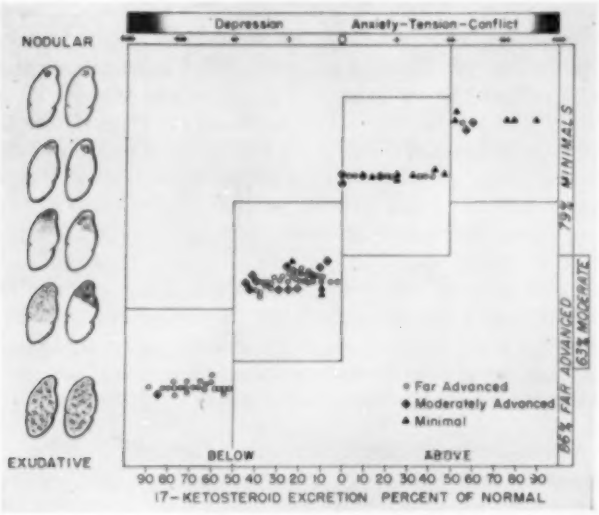


FIG. 1. The relation of 17-ketosteroid excretion to emotional state and extent and character of disease in pulmonary tuberculosis.

that which may be expected in a healthy person of the same age and sex. Consequently, the excretion observed for each patient was compared with the mean excretion expected in a healthy subject of the same age and sex, and the observed excretion has been expressed as per cent of the expected output for each patient. For this purpose, the mean data used were those provided by Kenigsberg and associates (35) from their study of 160 healthy subjects by essentially the same method.

The findings obtained from the *initial* study of each pulmonary case in the present series are shown in figure 1, in which observed excretion has been plotted as per cent of "normal" expected for age and sex. These initial data indicate that 17-ketosteroid excretion in the pulmonary patients ranged from 88 per cent below to 168 per cent above the mean excretion expected for healthy persons.

A study of the extent and character of the disease, as shown by roentgenography in each case, reveals that patients with greatly reduced 17-ketosteroid excretion generally have frankly exudative disease involving all of one lung or most of both lungs. Patients with less reduction of 17-ketosteroid excretion have exudative but somewhat less extensive disease, often involving only a part of one or both lungs. The disease process was noted to be more localized in extent and less exudative in nature as 17-ketosteroid excretion approached the "normal" mean. Patients with 17-ketosteroid output near the mean expected for age and sex usually had minimal disease of mixed type and, as a general rule, those patients with output above the normal had well-localized, productive tuberculosis.

When the extent of disease was considered in the light of the standard classi-

fication of the National Tuberculosis Association, it was found that 86 per cent of the far advanced patients had 17-ketosteroid excretion below the normal mean and 37 per cent of the far advanced patients studied had a 17-ketosteroid output more than 50 per cent below normal. Sixty-three per cent of the moderately advanced group had a reduction in 17-ketosteroid excretion of less than 50 per cent. Seventy-nine per cent of the patients with minimal pulmonary disease were above the normal mean. The remainder of the minimal cases had a 17-ketosteroid excretion below normal by less than 25 per cent.

From these data it is evident that there is a general correlation between extent and character of disease and 17-ketosteroid excretion. These findings establish a relationship between severity of disease and 17-ketosteroid excretion and are in general agreement in this respect with the findings of others (39–43).

Exceptions to this general relationship are evident, however, and suggest that factors other than severity of disease were also influencing 17-ketosteroid excretion at the time of the initial study.

17-Ketosteroid Excretion Versus Emotional State

From careful clinical observations of emotional state during the twenty-four-hour period of urine collection, it has been possible to establish a consistent relationship with 17-ketosteroid excretion during that time period. The relationship observed at the initial study is also illustrated in figure 1. These patients exhibited a spectrum of emotional behavior which ranged from frank depression and withdrawal at the lowest levels of 17-ketosteroid excretion to overt anxiety and preparation for action at the highest levels.

The patients with 17-ketosteroid excretion which was 50 per cent or more below normal were apathetic, withdrawn, occasionally confused, and preferred to be left alone. The patients with a less severe reduction of 17-ketosteroid excretion were irritable, uninterested in ward activities, and often lethargic. They also demonstrated a passive acceptance of bed rest. Those patients with slight reduction in 17-ketosteroid excretion were superficially calm, contented to be in the hospital, enjoyed the service they received, and, in general, appeared well adjusted to their situation. The patients with levels of excretion slightly above the normal were alert, cooperative, and interested in ward activity. This group enjoyed occupational therapy and used acceptable techniques to occupy their time.

The classical signs of anxiety, such as tachycardia, transient hypertension, insomnia, tremor of the hands, and palmar sweating, were the hallmark of the patient with 17-ketosteroid excretion substantially above the normal mean. Those patients with very high levels of excretion had these signs but were also restless, fearful, hostile, and unable to stay in bed.

The distribution of patients according to emotional state and extent of disease, as shown in figure 1, is based upon the 17-ketosteroid excretion found on admission to the study. It is to be expected that the emotional state of the patient following admission might be influenced by a change from the familiar environment at home to the environment of the hospital, with which few patients have had previous experience. Any threat imposed by treatment techniques or other

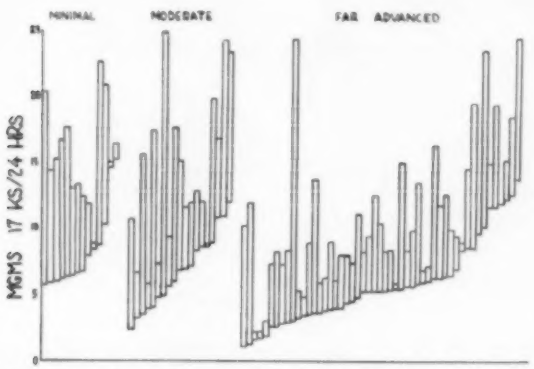


FIG. 2. The range of 24-hour 17-ketosteroid excretion in the cases of pulmonary tuberculosis by stage of disease.

events in the course of daily life in a tuberculosis hospital may be expected to influence 17-ketosteroid excretion by inducing a change in emotional state. Such emotional changes have been observed in all of the patients studied and have been promptly followed by changes in 17-ketosteroid excretion. This relationship is illustrated in figures 3 through 7.

The magnitude of change in 17-ketosteroid excretion resulting from change in emotional status which was observed in the 92 pulmonary cases is shown in figure 2. The patients have been grouped according to extent of disease, and the magnitude of change in 17-ketosteroid excretion has been graphed according to the lowest level of excretion observed at any time during the period of study. From this observation it is evident that 17-ketosteroid excretion may undergo change of considerable magnitude in response to change in emotional state induced by various events which transpire during the course of hospitalization.

The duration of change in 17-ketosteroid excretion was consistent with the duration of emotional response. It was transient in acute stress situations but was observed to persist for months in a setting of chronic stress (*see* figures 3 through 7).

Changes in 17-Ketosteroid Excretion with Time and the Course of Tuberculosis

The changes in 17-ketosteroid excretion observed with the passage of time were widely variable from patient to patient, as might be expected in light of the influence of emotional state upon 17-ketosteroid output. Nevertheless, certain trends became apparent with prolonged study.

Some patients on admission to the hospital were found to have excretion levels above, at, or below the normal level expected for age and sex which remained essentially stationary during prolonged observation. Other patients had high or low initial excretion levels which changed in the direction of normal over varying intervals of time. A third group exhibited a fluctuating pattern of excretion which might or might not approach normal from time to time.

TABLE 2

COURSE OF TUBERCULOSIS IN RELATION TO 17-KETOSTEROID EXCRETION

COURSE OF DISEASE	COURSE OF KETOSTEROID EXCRETION			
	Stationary near normal	Changing toward normal	Stationary above or below	Widely fluctuating
Moderate or rapid improvement	8	36	6	5
Slight or slow improvement	1	9	20	2
Stationary	1	2	7	1
Worse		1	5	1
Dead			3	1

From careful comparison of the course of tuberculosis (as judged from serial chest roentgenograms or laboratory data such as sputum or cerebrospinal fluid findings) with the observed pattern of 17-ketosteroid excretion, it was possible to establish a correlation between these two factors. The results of such comparison are shown in table 2.

In general, the tuberculosis was noted to improve in a gratifying manner in those patients whose 17-ketosteroid excretion was approaching or was already near the normal excretion expected for age and sex. The tuberculosis was found to improve slowly or not at all in those patients whose excretion remained at some distance above or below the normal. Patients with fluctuating excretion exhibited improvement in relation, not only to the proximity of their excretion to normal, but to the time during which this condition pertained. Ketosteroid excretion was decreasing when worsening of the disease or death occurred. It is interesting to note that microscopic study of the adrenal glands obtained from 4 patients at necropsy failed to demonstrate significant structural change except in one case of miliary tuberculosis, in which a few miliary tubercles but no extensive destruction of the gland were noted.

ILLUSTRATIVE CASES

In figures 3 through 7, typical cases from the series studied are illustrated, and the parallel between emotional state, 17-ketosteroid excretion, and the course of tuberculosis during the period of study are demonstrated.

Case 1 (Figures 3*A*, *B*, *C*). E. A., a 57-year-old white male laborer and chronic alcoholic, was admitted to the hospital on December 27, 1951, with minimal tuberculosis, mixed in character, in the right apex and a small productive lesion behind the left first rib. Sputum was negative for *M. tuberculosis* by smear and culture until February 29, 1952, when one smear positive for acid-fast bacilli was obtained. On treatment with bed rest, the right apical lesion had become a stable nodule by May, 1952. The patient accepted bed rest poorly and was somewhat uncooperative. On September 23, 1952, in preparation for nodulectomy, the patient was started on combined chemotherapy with streptomycin–para-aminosalicylic acid (PAS). Tubercle bacilli isolated on November 25, 1952, revealed significant resistance to streptomycin. The patient became discouraged over the long hospitalization, refused further chemotherapy, became intoxicated on the open ward, and was removed to the detention ward. He became increasingly depressed, and on February 24, 1953, the sputum was positive on smear.

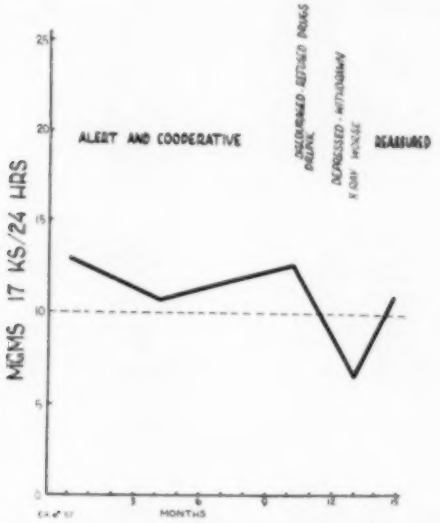


FIG. 3A. *Case 1* (E. A). Changes in emotional state and 17-ketosteroid excretion during treatment.

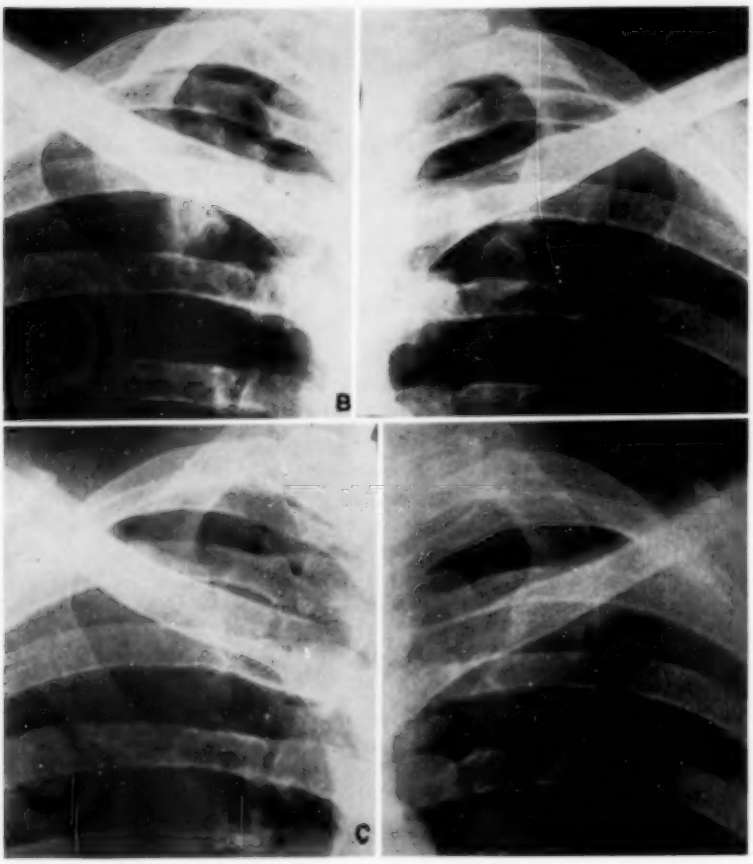
At this time, on roentgenographic examination, an exudative lesion was noted at the site of the previous lesion in the left apex. The patient was released from the detention ward and was started on isoniazid-PAS in an effort to control the disease.

This patient exhibited prompt control of a small active lesion during a period of 17-ketosteroid excretion slightly above the normal level, with worsening of the disease at a time in his hospitalization when he was under no effective chemotherapy and had reduced 17-ketosteroid excretion accompanied by a moderate depression.

Case 2 (Figures 4*A*, *B*, *C*). L. O. K., a 42-year-old white male, was admitted to the hospital on October 24, 1952, with far advanced bilateral exudative disease and sputum positive for *acid-fast bacilli* on smear. He was acutely ill, febrile, and underweight, but alert and cooperative. Treatment with streptomycin-PAS was started immediately after admission. The chest roetgenogram demonstrated minimal improvement by December 17, 1952, and has since remained unchanged in spite of a pneumoperitoneum and the addition of isoniazid on March 3, 1953. The patient became progressively more discouraged. This attitude was accompanied by a decreasing 17-ketosteroid excretion. (The temporary encouragement offered by the institution of isoniazid therapy was accompanied by a slight increase in excretion in March, 1953.)

This patient illustrates the unsatisfactory course of the disease which accompanies a 17-ketosteroid excretion change in the direction away from normal.

Case 3 (Figures 5*A*, *B*, *C*). J. S., a 42-year-old white male steelworker, was admitted to the hospital on November 20, 1951, with far advanced bilateral tuberculosis, mixed in type, and complicated by tuberculous laryngitis and enteritis. At the time of admission the patient was acutely ill, febrile, and moderately underweight. He had entered the hospital against



FIGS. 3*B* and *C*. *Case 1* (E. A.)

B. Roentgenogram at start of study (December 18, 1951), showing a mixed type of minimal lesion behind the anterior end of the right first rib.

C. Roentgenogram at end of study (March 23, 1953), showing the improvement in the lesion on the right and a new exudative lesion in the left first interspace.

the wishes of his wife, who was a faith-healer, and was tense and apprehensive, with many of the clinical signs of anxiety. Treatment with bed rest and streptomycin-PAS was instituted immediately, even though the patient was fearful of toxic effects from these agents and his wife was bitterly opposed to their use. During the first seven months of treatment no significant change in disease other than slow cavity closure was noted on serial roentgenograms. Sputum remained positive for *M. tuberculosis* on microscopic examination and culture. During these months the patient was continually plagued by disagreement with his wife regarding his treatment and continuously threatened by a serious financial situation at home. He required frequent leaves and was often found out of bed against advice.

In June, 1952, coincident with the solution of some of his problems, the patient became less anxious and restless, accepted bed rest more willingly, and "converted" his sputum by

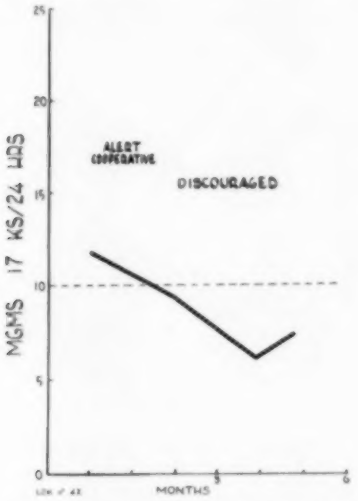
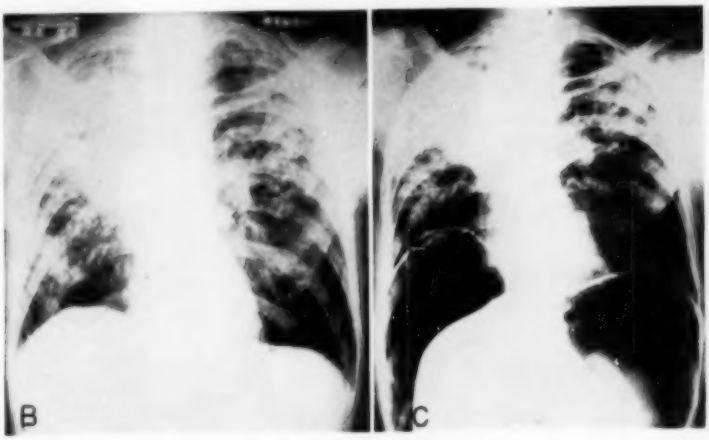


FIG. 4A. *Case 2* (L. O. K.). Changes in emotional state and 17-ketosteroid excretion during treatment.



FIGS. 4B and C. *Case 2* (L. O. K.)

B. Roentgenogram at start of study (October 27, 1952), showing extensive bilateral, exudative, and cavitary tuberculosis.

C. Roentgenogram at end of study (March 23, 1953), showing only minimal improvement bilaterally despite five months of intensive chemotherapy and pneumoperitoneum.

culture on July 22, 1952. He continued superficially calm and cooperative, with some clearing of disease by roentgenography until February, 1953, when a poorly positioned chest film suggested a possible increase in disease. Some delay in confirming this change was accompanied by a return of all of the previous signs of apprehension and anxiety and a return to his earlier type of behavior. He refused further treatment with streptomycin because it

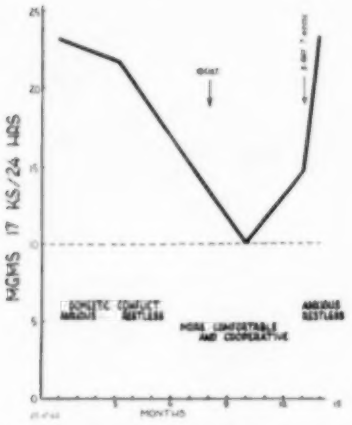
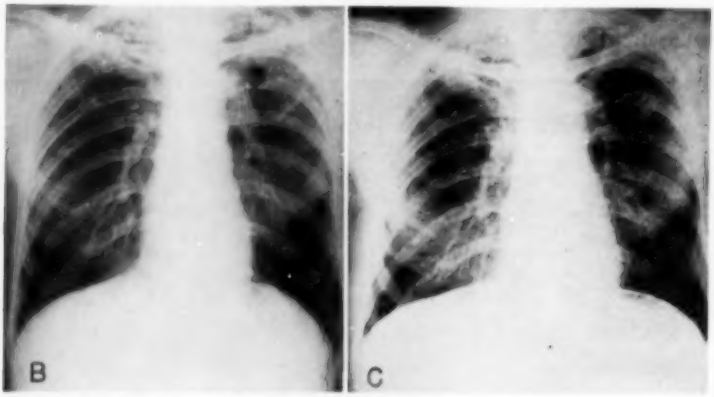


FIG. 5A. *Case 3* (J. S.). Changes in emotional state and 17-ketosteroid excretion during treatment.



FIGS. 5B and C. *Case 3* (J. S.)

B. Roentgenogram at start of study (November 21, 1951), showing mixed-type, far advanced tuberculosis.

C. Roentgenogram at end of study (February 25, 1953), showing minimal improvement in the disease process after fifteen months of treatment.

caused swelling of the face and lips. Isoniazid had to be substituted. Recent chest roentgenograms have failed to confirm a worsening of disease but also fail to show further improvement. The patient is currently being evaluated for resectional surgery and is overtly restless and apprehensive.

This patient illustrates the high 17-ketosteroid excretion which accompanies severe anxiety and demonstrates the slow response to treatment (despite chemotherapy) noted in such circumstances.

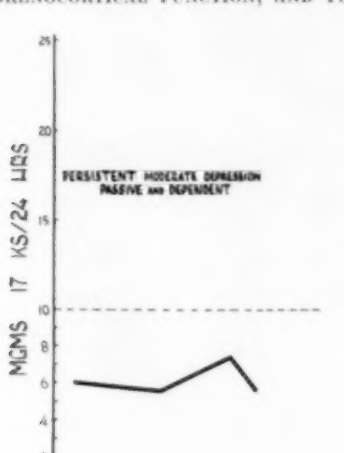
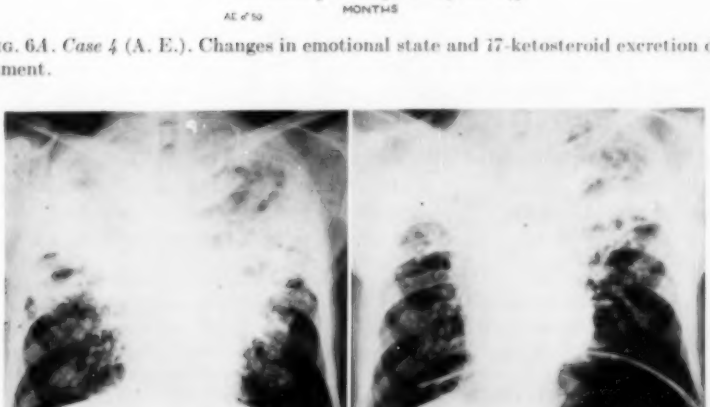


FIG. 6A. *Case 4* (A. E.). Changes in emotional state and 17-ketosteroid excretion during treatment.



FIGS. 6*B* and *C*. *Case 4* (A. E.)

B. Roentgenogram at start of study (June 16, 1952), showing very extensive, bilateral, exudative, and cavitary tuberculosis.

C. Roentgenogram at end of study (January 19, 1953), showing minimal improvement after seven months of chemotherapy and pneumoperitoneum.

Case 4 (Figures 6*A*, *B*, *C*). A. E., a 50-year-old white male laborer, was admitted to the hospital on June 4, 1952, with far advanced, bilateral, exudative, and cavitary tuberculosis and sputum positive for acid-fast bacilli on smear. He has been treated continuously since admission with bed rest and isoniazid-PAS. On admission this patient was acutely and

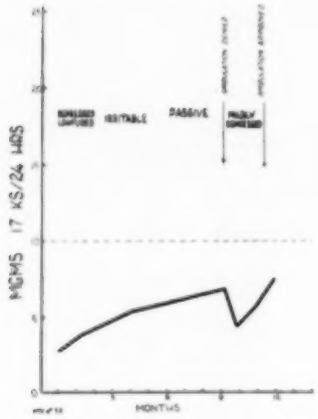


FIG. 7A. *Case 5* (H. N.). Changes in emotional state and 17-ketosteroid excretion during treatment.

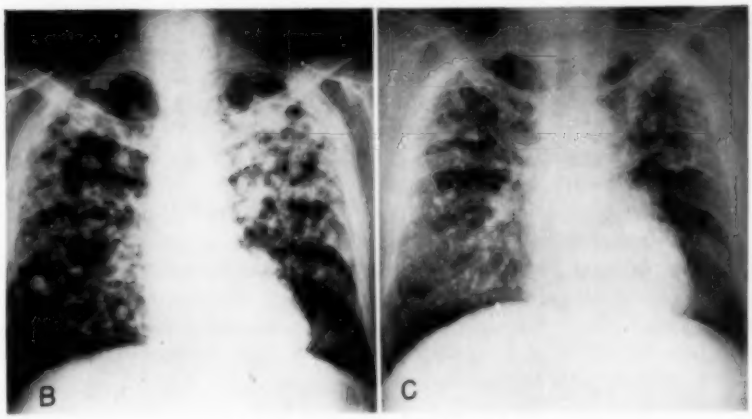
chronically ill, febrile, and moderately underweight. He was passive, dependent, and willing to accept hospital care. He has shown persistent chronic depression, with periods of irritability, and has required excessive medication for cough and sleep throughout the period of treatment.

This patient illustrates the lowered ketosteroid excretion which accompanies chronic psychic depression. The poor response of tuberculosis (in spite of chemotherapy) during this period is also evident.

Case 5 (Figures 7*A*, *B*, *C*). H. N., a 52-year-old white male bartender, was admitted to the hospital on March 12, 1952, with far advanced, bilateral exudative, and cavitary tuberculosis and sputum positive for acid-fast bacilli on microscopic examination. At the time of admission this patient was acutely ill, febrile, cachectic, overtly depressed, and profoundly weak. Treatment with streptomycin (1 gm. daily) and PAS was started immediately. During the first month of treatment the patient's clinical state deteriorated. During the second month of treatment, he became agitated and difficult to manage. On April 24, 1952, isoniazid was substituted for PAS. The patient continued to show alternating periods of confusion and irrational behavior, with periods of hallucination, until early June, 1952. About this time the patient began to show gradually increasing irritability with episodes of frank anger aimed at the nurses. This behavior slowly gave way to more permissive and accepting behavior. By July, 1952, repeated gastric specimens were negative for *M. tuberculosis* on culture. Throughout this entire period, serial chest roentgenograms at monthly intervals revealed steady improvement in the visible disease.

Because of moderately severe joint stiffness and pain, the patient requested bathroom privileges in late November, 1952. These were denied, and the patient subsequently became moderately depressed. During this period no further improvement was noted by roentgenography. Ambulation was approved on December 30, 1952, and since that time has been slowly and progressively carried out. Subsequent roentgenograms have shown continuing improvement.

This patient illustrates the change in ketosteroid excretion toward normal which is associated with steady improvement of tuberculosis during treatment.



FIGS. 7*B* and *C*. *Case 5* (H. N.)

B. Roentgenogram at start of study (March 13, 1952), showing far advanced, bilateral, exudative, and cavitary tuberculosis.

C. Roentgenogram at end of study (March 19, 1953), showing clearing of disease and development of fibrosis after one year of chemotherapy.

His case also demonstrates the cessation of improvement which has been noted to occur coincident with a sudden change in ketosteroid excretion induced by a change in emotional state.

It is interesting to note the reabsorption of exudative lesions with minimal fibrosis and nodulation which this patient's case demonstrates in contrast to the type of change seen in *Cases 1* and *3*, in which fibrosis was distinctly evident.

DISCUSSION

It is recognized that 17-ketosteroid substances or their precursors arise from both adrenal and testicular sources (37, 38). It is also acknowledged that the metabolism of these substances prior to excretion by the kidney may be subject to influence by certain conditions (26, 27, 45). Nevertheless, in the absence of a single, complete, and quantitative method of measurement, the urinary 17-ketosteroid excretion has been considered to be the best over-all index of adrenocortical function (44). For this reason, as well as for reasons of simplicity and availability, the method was chosen for use in the present study.

The mean 17-ketosteroid excretion found in the 84 males and 25 females studied indicates a significant alteration of adrenocortical function in tuberculous patients when considered in relation to the mean excretion of 16 and 9.3 mg. per twenty-four hours, respectively, in healthy men and women. When due allowance is made for the effect of age upon ketosteroid excretion, a consistent alteration of adrenocortical function by age groups is evident in these patients.

The present findings are in agreement with those of others (39, 40, 41, 42, 43). Bastenie and Kowalewski (42) have suggested that the alteration of adrenocor-

tical function in clinical tuberculosis is related to the severity of the disease. The data given by Rivoire and associates (43) also indicate such a relationship. In general, the present data concerning the correlation of extent of the disease to ketosteroid excretion are in agreement with this concept. Nevertheless, the fact that approximately one-third of the present patients had normal or high mean excretion levels suggests that factors other than severity of the disease are also influencing adrenocortical activity.

The influence of emotional status upon adrenocortical activity, as measured by urinary 17-ketosteroid excretion and other methods, is well established from the studies of many investigators (16–30). The demonstration in the present study of consistent and significant alterations in 17-ketosteroid excretion in tuberculous patients in response to the stress of emotional conflict and threatening life situations is in agreement with the findings in nontuberculous subjects reported by these other investigators.

The possibility that changes in adrenocortical function induced by such stress may influence the course of tuberculosis may be inferred from the parallel between emotional state, ketosteroid excretion, and response of tuberculosis during treatment, as illustrated in figures 3 through 7.

The various studies of the effects of cortisone upon experimental tuberculosis are quite conflicting and appear "to depend to a considerable extent upon the precise details of the experiment" (55). In certain circumstances, deleterious or potentially deleterious effects upon resistance have been observed (4, 5, 7, 11). In different experimental circumstances, other investigators (9, 52, 53) have been unable to demonstrate any significant effect with pharmacologic doses of cortisone.

With the exception of the results obtained by Bunn and Drobeck (8), no data are available to suggest that a deleterious effect upon resistance may be induced by corticotropin. In fact, Lurie and co-workers (53) suggest that the resistance of certain animals may be increased by corticotropin.

LeMaistre and associates (1) have demonstrated that the short-term administration of cortisone and corticotropin to patients induces a temporary amelioration of the signs and symptoms of acute illness and a regression of certain features of laryngeal tuberculous lesions. These workers noted a prompt return of the signs and symptoms of illness upon withdrawal of the hormone. Wallner and associates (3) confirmed the symptomatic improvement but were unable to demonstrate improvement in laryngeal lesions.

Although these various studies seem to indicate that experimental conditions (such as the amount and frequency of hormone administered, certain factors of host-parasite relationship, and the time during which observations were made) influence the results, they also suggest that under certain conditions pharmacologically induced hyper-adrenocorticism may alter host mechanisms involved in resistance to infection.

The studies of Cushing (46), Perla (47), Steinbach and associates (48), and Tonutti and Fetzer (49) in experimental animals suggest that experimentally induced states of hypo-adrenocorticism may affect resistance to tuberculosis

adversely. The studies of Lurie and associates (4, 50) and Ebert (15) provide some understanding of the mechanisms by which adrenal hormones may alter bodily defense against tubercle bacilli.

In the present study, the correlations established between the course of tuberculosis and changes in ketosteroid excretion during treatment suggest that endogenous adrenocortical hormones in high or low concentration may influence resistance as well.

Bunn and Drobeck (8) have shown that experimental infection with tubercle bacilli *after* a state of hyper-adrenocorticism has been induced fails to incite significant tissue response as long as that state is maintained. If hypo-adrenocorticism ensues as in the "rebound" following hormone withdrawal (1), however, prompt tissue response occurs.

This finding, together with the present observations, leads to the postulation that, in man, states of hyper-adrenocortical function are consistent with resistance to tuberculosis as long as that state persists. Following infection, if hypo-adrenocorticism ensues as a result of chronic stress, the typical tissue response of tuberculosis promptly appears. This hypothesis is suggested as a basis for further investigation.

SUMMARY

A study of adrenocortical function has been made in 25 female and 84 male patients with various types of tuberculosis, using 17-ketosteroids as the index of such function.

From the data obtained, it is concluded that a significant reduction of 17-ketosteroid excretion is present in tuberculosis.

When a correlation is attempted between 17-ketosteroid excretion and extent of the disease, it becomes evident that, as a general rule, low 17-ketosteroid excretion is associated with extensive and exudative tuberculosis and that high 17-ketosteroid excretion is associated with fibrotic tuberculosis of limited extent.

It has been shown that 17-ketosteroid excretion in the cases studied is also influenced by the emotional state of the patient.

From the observed parallel between emotional state, 17-ketosteroid excretion, and course of tuberculosis during treatment, it is inferred that emotional states resulting from a variety of life-situations are influencing adrenocortical function and thereby affecting the response of tuberculosis during treatment.

A working hypothesis is suggested as a basis for further investigation.

SUMARIO

La Relación entre la Tensión, la Función Suprarrenocortical y la Tuberculosis

Verificóse un estudio de la función corticosuprarrenal en 25 mujeres y 84 varones que tenían varias formas de tuberculosis, usando 17-quetosteroides como índice de dicha función.

Por los datos obtenidos, se deduce que en la tuberculosis existe una importante reducción de la excreción de 17-quetosteroides.

Cuando se trata de establecer una correlación entre la excreción de 17-quetosteroides

y la extensión de la dolencia, se vuelve manifiesto que, por regla general, una excreción baja de quetosteroides se asocia con tuberculosis extensa y exudativa y una excreción alta con tuberculosis fibrosa en grado moderado.

Quedó demostrado que la excreción de 17-quetosteroides en los casos estudiados se ve además afectada por el estado afectivo del enfermo.

A la luz del paralelo observado entre el estado afectivo, la excreción de 17-quetosteroides y la evolución de la tuberculosis durante el tratamiento, infiérese que los estados afectivos provenientes de varias situaciones en la vida afectan la función corticosuprarrenal, modificando así la respuesta de la tuberculosis durante el tratamiento.

Ofrécese una hipótesis de trabajo como base para investigaciones ulteriores.

RESUME

Les relations entre le "stress", la fonction cortico-surrénale et la tuberculose

Une étude de la fonction cortico-surrénale a été effectuée chez 25 femmes et 84 hommes présentant différents types de tuberculose, en prenant comme indice de cette fonction les 17-cétostéroïdes.

D'après les données obtenues, les auteurs concluent qu'il y a une réduction significative de l'excrétion des 17-cétostéroïdes dans la tuberculose.

Quand on essaye d'établir une corrélation entre l'excrétion des 17-cétostéroïdes et l'étendue de l'affection, il devient évident que, d'une manière générale, l'excrétion faible des 17-cétostéroïdes s'accompagne de tuberculose extensive et exudative et que l'excrétion élevée des 17-cétostéroïdes est associée, dans une certaine mesure, à la tuberculose de forme fibreuse.

Dans les cas étudiés on a observé que l'excrétion des 17-cétostéroïdes était également influencée par l'état émotionnel du patient.

A la suite du parallélisme observé entre l'état émotionnel, l'excrétion des 17-cétostéroïdes et l'évolution de la tuberculose au cours du traitement, les auteurs supposent que l'état émotionnel engendré par diverses situations de la vie influence la fonction cortico-surrénale, et par celà même affecte la réponse de la tuberculose durant le traitement.

Une investigation fondée sur cette hypothèse est proposée par les auteurs.

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THE TREATMENT OF TUBERCULOUS MENINGITIS[1]

A Study of Forty-Six Adult Patients

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INTRODUCTION

In the seven years that have elapsed since the first clinical use of streptomycin in the treatment of tuberculous meningitis, repeated studies have demonstrated the ability of this drug to influence favorably the course of this infection. Prior to the discovery of streptomycin, meningeal tuberculosis ran a rapid and fatal course. With the aid of this new and potent drug, many lives were prolonged, meningeal infection was apparently controlled, and a few fortunate patients were returned to their normal ways of life.

Shortly after the introduction of streptomycin, numerous studies on the human absorption, distribution, and excretion of this new substance were performed. The diffusion of parenterally administered streptomycin across an inflamed meningeal surface into the cerebrospinal fluid in therapeutic quantities was well established (1, 2, 3).

The present report is concerned with a study of 46 consecutively treated adult patients with tuberculous meningitis, studied on the Chest Service at Fitzsimons Army Hospital during the five-year period from 1948 to 1953. The study was originally undertaken primarily to evaluate the role of parenteral streptomycin in the treatment of this disease in the adult patient. While pursuing this study, concepts dealing with the total duration of successful chemotherapy and the proper combination of drugs gradually evolved which greatly modified the original scope of this study.

MATERIALS AND METHODS

The 46 patients studied were derived from three principal groups. The majority were members of the Armed Forces of the United States, with smaller groups comprised of civilian dependents and beneficiaries of the Veterans Administration.

There were 39 males and 7 females. Twenty-nine patients were white, 15 were Negroes, and 2 were American Indians. Ages ranged from seventeen to fifty-six years, with an average of twenty-seven years. Seventy per cent of the group were between the ages of eighteen and thirty years. Of these, the largest fraction fell into the group of twenty-one to twenty-five years.

To a certain extent this group of patients was selected, in that many of them were transported great distances to this hospital for treatment of tuberculous meningitis. The patients were studied on designated research wards, and all laboratory studies were performed by one laboratory.

In addition to the routine laboratory studies, complete cerebrospinal fluid examinations were performed monthly. These included culture for tubercle bacilli and quantitative

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biologic assay for the drugs which were employed orally and parenterally. When fully recovered, all patients were repeatedly examined by the Department of Otolaryngology for evidence of eighth-nerve damage. Hearing loss was recorded by audiometric study. Labyrinthine function was evaluated by the Bárány caloric test. Post-mortem studies were performed by the Department of Pathology on 18 of the 19 patients who died.

Diagnosis

The diagnosis of meningeal tuberculosis was established by culturing *M. tuberculosis* from the cerebrospinal fluid in 14 patients (30 per cent). In 6 additional patients, acid-fast organisms were demonstrated on smear of the pellicle formed in the cerebrospinal fluid. In the remaining 26 patients, the diagnosis was presumed by the presence of bacteriologically proved tuberculosis elsewhere, coupled with cerebrospinal fluid findings of a nonpurulent meningitis, with a sugar content below 35 mg. per 100 ml. of cerebrospinal fluid. Autopsy studies performed on the treatment failures in the latter group have confirmed the validity of the clinical diagnosis in each instance.

Of the 46 patients, 21 (45 per cent) had coexistent miliary tuberculosis. In addition, the following tuberculous complications were present: 10 patients had chronic pulmonary tuberculosis; 9 had urogenital tuberculosis; 9 had pleural effusions; 2 had tuberculous spondylitis; and one had massive generalized tuberculous lymphadenitis.

Treatment Regimens

Ten of the 46 patients received combined intrathecal and parenteral streptomycin at other hospitals prior to transfer to Fitzsimons Army Hospital. Doses of streptomycin employed intrathecally varied from 50 mg. three times weekly to 100 mg. daily, and the duration of intrathecal treatment ranged from one to fifteen weeks, averaging five weeks. On admission to this hospital, intrathecal streptomycin was discontinued in all 10 patients, but the parenteral and oral administration of chemotherapeutic agents was continued. The results of treatment in this group of 10 patients will be considered separately from the larger series. The remaining 36 patients comprise the group whose treatment was started at Fitzsimons Army Hospital and who received no intrathecal streptomycin.

There have been four different programs of drug treatment employed in the present study, reflecting closely the introduction of new drugs and the changing concepts in tuberculosis chemotherapy within the past five years.

Regimen 1: Streptomycin was the only available agent for the earliest patients. This drug was given parenterally in daily, divided doses totaling 2 to 3 gm. per day for a period of four weeks. A "rest period" of two weeks without any chemotherapy then followed in an effort to prevent the emergence of resistant organisms. Thereafter, parenteral streptomycin was resumed in the above dosage and given for another four-week period, again followed by a "rest period"; this scheme was followed until the cerebrospinal fluid findings returned to normal. There were 6 patients treated in this manner.

Regimen 2: Following the development and successful clinical use of para-aminosalicylic acid (PAS), the above program was revised. New patients were then started on 2 to 3 gm. daily of parenteral streptomycin and 12 gm. of PAS by mouth daily for a period of four weeks. Thereafter, four-week courses of daily streptomycin were alternated with four-week courses of oral PAS. When necessary, intravenous or subcutaneous administration of the sodium salt of PAS was employed.

During this time the benefits of prolonged chemotherapy gradually became evident, and the danger of relapse following short courses of treatment was recognized. Therefore, the treatment of tuberculous meningitis was prolonged to include a period of at

least six months after the cerebrospinal fluid findings had returned to normal. This meant that most patients received continuous chemotherapy for a period of at least twelve months. The largest of the four treatment groups, 22 patients in all, received combined streptomycin-PAS treatment.

Regimen 3: In 1951, viomycin[3] was added to the study, and this led to another revision of treatment. New patients were placed on oral PAS, 12 gm. daily for the duration of treatment, and in addition received four-week courses of streptomycin, 2 to 3 gm. daily, alternating with four-week courses of 1 to 2 gm. of parenteral viomycin daily. Again, all drugs were continued for six additional months after the cerebrospinal fluid findings had returned to normal. Seven patients were treated on this regimen.

Regimen 4: Isoniazid next appeared as a potent agent in the treatment of tuberculosis and was quickly added to the program. Earlier studies had demonstrated the ability of this drug to enter the cerebrospinal fluid in therapeutically effective quantities (4). A later report confirmed its beneficial effect in the treatment of tuberculous meningitis (5). The program of treatment at this hospital was again changed to one embodying triple daily administration of streptomycin, isoniazid, and PAS. As experience accumulated with the use of isoniazid, its total daily dose was increased from 4 to 5 mg. per kg. of body weight to 9 to 10 mg. per kg. of body weight.

For the past year the following has been the standard treatment of meningeal tuberculosis employed at this hospital. Streptomycin, 2 gm. daily, in divided parenteral doses is given for a period of two months. The dose is then reduced to 1 gm. daily and continued until the cerebrospinal fluid findings have returned to normal. Thereafter, it is continued in intermittent dosage of 1 gm. every third day for a period of at least six additional months. Isoniazid and PAS are given concurrently, the former in divided oral doses totaling 600 to 750 mg. daily, and the latter orally, 12 gm. daily. Eleven patients have been started on this newest plan of treatment. Whenever indicated by the presence of serious extrameningeal tuberculosis, chemotherapy is extended beyond the six-month period following the return of the cerebrospinal fluid findings to normal.

Following completion of chemotherapy, all patients were observed for a period of at least six months on a program of progressive ambulation and vocational rehabilitation prior to discharge from the hospital.

RESULTS

In the five-year period, 19 (41 per cent) of 46 patients died, all while receiving drug treatment. Of the 27 survivors, 10 patients (21 per cent) were discharged as recovered. All of these recoveries preceded the clinical use of isoniazid. The period of hospitalization in this group ranged from 14 to 50 months, and averaged 23 months. The duration of chemotherapy received by these 10 patients ranged from 12 to 28 months and averaged 17.3 months. Follow-up studies on 8 of this group of patients, averaging 21 months after discharge, revealed no evidence of a relapse of the meningeal infection. Of the 17 patients still hospitalized, 12 (21 per cent) have been treated to the point of a normal cerebrospinal

[3] Supplied by Charles Pfizer & Co., Inc., Brooklyn, New York.

TABLE 1

SURVIVAL WITH INTRATHECAL STREPTOMYCIN FOLLOWED BY PARENTERAL THERAPY

AGE, RACE	INTRATHECAL STREPTOMYCIN	SURVIVAL FOLLOWING INTRATHECAL STREPTOMYCIN	TOTAL LENGTH OF ALL CHEMOTHERAPY	REGIMEN USED AT FITZSIMONS ARMY HOSPITAL	FOLLOW-UP
	weeks		*months*		
1. 21, N	4	4 weeks	2	SM—PAS	Died—2 months
2. 22, N	1	7 weeks	2	SM—PAS	Died—2 months
3. 21, W	2	3 months	3.5	SM—PAS	Died— 3.5 months
4. 22, W	4	12 months	13	SM—PAS	Died—13 months
5. 31, W	16	32 weeks	12	SM	Died—12 months
6. 21, W	4	4 years	13	SM	L & W— 4 years
7. 21, W	4	4 years	27	SM—PAS	L & W— 4 years
8. 20, N	2	2 years	13	SM—VM—PAS	L & W— 2 years
9. 22, IND	12	1.5 years	14	SM—PAS	L & W— 1.5 years
10. 22, N	2	2 years	15	SM—VM—PAS	L & W—2 years

N—Negro, W—White, IND—American Indian, SM—Streptomycin, VM—Viomycin, L & W—Living and Well.

fluid and are expected to recover from this episode of meningitis. The 5 remaining patients are responding in a satisfactory manner to treatment.

Intrathecal administration of streptomycin: Ten patients previously mentioned were treated with intrathecal and parenteral streptomycin at other military hospitals prior to transfer to Fitzsimons Army Hospital. On admission to this hospital, intrathecal streptomycin was discontinued but oral and parenteral drug therapy continued as outlined above. Five of this group died, and 5 survive to date. All 5 treatment failures were in patients who did not have concomitant miliary tuberculosis. Of the 5 survivors, 3 have been discharged as recovered. The remaining 2 patients have normal cerebrospinal fluid findings, have completed the program of drug treatment, and will be discharged shortly. The length of survival and duration of chemotherapy in this group are shown in table 1.

In this small group, survival did not appear to be related to either the age or the race of the patient or to the duration of intrathecal treatment. Clinically, survival did not correlate with the presence or extent of recognizable extrameningeal tuberculosis.

The remaining 36 patients with meningeal tuberculosis did not receive intrathecal streptomycin. Of this number, 14 (38 per cent) have died and 22 survive, at least four months after start of treatment. Six of 16 patients (37 per cent) without miliary tuberculosis and 8 of 20 patients (40 per cent) with concomitant miliary tuberculosis died. In this series, the presence of concomitant miliary tuberculosis did not appreciably alter mortality, in contrast to the findings of others (6).

Of the 22 survivors, 7 have been discharged and another 10 patients currently hospitalized have been treated to the extent that their cerebrospinal fluid findings are normal and recovery is expected.

TABLE 2

ANALYSIS OF TREATMENT FAILURES

AGE, RACE	CLINICAL TUBERCULOSIS PRECEDING MENINGITIS	MILIARY WITH MENINGITIS	SURVIVAL ON TREATMENT	REGIMEN
1. 21, W	Urogenital, 6 months	0	11 months	SM—PAS
2. 30, W	Miliary, 5 years	0	12 months	SM—PAS
3. 18, W	0, 0	0	2 months	SM—INH—PAS
4. 56, W	Pulmonary, Urogenital } 1 year	0	4 months	SM—PAS
5. 20, W	0, 0	0	6 days	SM
6. 40, W	Pulmonary, 2 years Urogenital, 1 year	0	4 months	SM—PAS
7. 28, W	0, 0	Yes	3 weeks	SM
8. 45, N	0, 0	Yes	1 week	SM—PAS
9. 32, N	Miliary, 9 months Urogenital, 6 months	Yes	5 months	SM—PAS
10. 53, W	0, 0	Yes	13 months	SM—PAS
11. 24, W	Lymphadenitis, 4 months	Yes	8 months	SM—PAS
12. 22, N	0, 0	Yes	2 months	SM—PAS
13. 31, W	Pulmonary, 7 years	Yes	2 months	SM
14. 19, N	0, 0	Yes	9 months	SM—VM—PAS

N—Negro, W—White, SM—Streptomycin, VM—Viomycin, INH—Isoniazid.

The pertinent data regarding the treatment failures in this group are given in table 2.

The period of survival from start of chemotherapy in this group ranged from 6 days to 13 months, and averaged 5.2 months. One half of this group did not have a clinical diagnosis of tuberculosis established prior to the onset of meningeal tuberculosis.

Ten patients have been discharged to date. Of these, 3 received two- to four-week courses of intrathecal streptomycin prior to prolonged chemotherapy by other routes at this hospital. The essential facts regarding the discharged patients are listed in table 3.

Damage to auditory apparatus: In 3 of the 10 discharged patents, there was evidence of moderate to severe damage to the eighth cranial nerve at the time of discharge. This has proved permanent, showing no tendency to either progress or diminish with the passage of time. It is of interest that none of these 3 received intrathecal streptomycin. It has not, however, proved incapacitating, as 2 of these men are gainfully employed. In these 10 patients, drug therapy was given for periods ranging from 12 to 28 months, with an average of 17.3 months.

An evaluation of the therapeutic efficiency of the four basic regimens employed in this study is presented in table 4.

Isoniazid: These data suggest that isoniazid has been of definite added value in the more rapid resolution of meningeal tuberculosis, as manifested by the clearing of the cerebrospinal fluid. The early defervescence, mental clearing, restoration of appetite, and rapid clearing of meningeal signs and symptoms which have been noted clinically are all indicative of a profound antibacterial action.

TABLE 3

ANALYSIS OF DISCHARGED PATIENTS

AGE, RACE	CLINICAL TUBERCULOSIS PRECEDING MENINGITIS	MILIARY WITH MENINGITIS	REGIMEN AND DURATION	RESIDUAL DEFECTS	KNOWN SURVIVAL
					years
1. 21, W	0, 0	0	Intrathecal SM, 1 month SM, 13 months	None	4
2. 21, W	0, 0	0	Intrathecal SM, 1 month SM—PAS, 21 months	None	4
3. 20, N	0, 0	0	Intrathecal SM, 2 weeks SM—PAS—VM, 12 months	None	2
4. 27, N	Pleural effusion; bilateral, 5 months				
	Tuberculous peritonitis, 4 months	0	SM, 14 months	++ hearing loss right ear, only	5
5. 22, N	0, 0	0	SM—PAS, 14 months	None	2
6. 21, W	0, 0	0	SM—PAS, 18 months	None	3
7. 32, N	Lumbar spine, 4 months				
	Pulmonary, 4 months	0	SM—PAS, 18 months	++ vestibular and hearing loss, bilateral	3
8. 39, W	Lumbar spine, 4 years				
			SM—PAS, 13 months	None	2
	Urogenital, 1 month	Yes			
9. 30, W	0, 0	Yes	SM—PAS, 28 months	++++ vestibular; ++ hearing loss, bilateral	5
10. 25, N	Urogenital, 4 months	Yes	SM—PAS, 18 months	None	3

W—White, N—Negro, SM—Streptomycin, VM—Viomycin.

In 11 previously untreated patients with tuberculous meningitis given large doses of isoniazid combined with daily parenteral streptomycin and oral PAS, there has been one death to date. This occurred in an 18-year-old, white pregnant female admitted in coma with untreated meningitis of two weeks' duration. She failed to regain consciousness and gradually lapsed into the picture of decerebrate rigidity, described by others (7) as invariably fatal. The patient died after two months of intensive treatment. In the 10 survivors, there has been a complete clearing of symptoms and clinical signs of meningitis during the first three months

TABLE 4

THERAPEUTIC EFFICIENCY OF REGIMENS

	SM	SM—PAS	SM—VM—PAS	SM—INH—PAS
Total number of patients	6	22	7	11
Dead	4	13	1	1
Discharged	2	7	1	0
Treated to normal cerebrospinal fluid	2	8	6	6
Average time for cerebrospinal fluid to become normal (months)	13.5	11	11.8	6.1

SM—Streptomycin, VM—Viomycin, INH—Isoniazid.

TABLE 5

RELATION OF EXTRAMENINGEAL DISEASE TO SURVIVAL

	TOTAL NUMBER	LIVING	DEAD	PER CENT MORTALITY
Genitourinary system	8	2	6	75
Bone	2	2	0	0
Pulmonary	9	6	3	33
Pleural	9	7	2	22

of treatment. Six have regained a normal cerebrospinal fluid, and prolonged treatment is being continued. The time required to regain a normal cerebrospinal fluid in these 6 patients ranged between four and eight months, and averaged 6 months.

Extrameningeal foci: There were 12 patients with meningeal tuberculosis who did not have other clinically recognized foci of active disease. In none of these patients was a hematogenous dissemination noted clinically. Of this number, 6 died and 6 survived. Autopsy studies on the 6 treatment failures revealed tuberculosis of the bronchopulmonary and mediastinal lymph nodes with a healed pulmonary parenchymal focus in 5, all undetected on routine chest roentgenograms. The remaining patient had tuberculosis of the retroperitoneal lymph nodes. In no instance were cortical tubercles identified on routine section of the brain in the 12 patients.

Of the 8 patients with combined miliary and meningeal tuberculosis who died, 3 had extensive, caseous, urogenital tuberculosis. In the remaining 5 patients, it was believed that the hematogenous dissemination had evolved from mediastinal and bronchopulmonary lymphadenitis, related to a primary pulmonary infection. Cortical tubercles were identified on routine section of the brain in 2 instances in this group.

The presence of clinically recognized urogenital tuberculosis carried the gravest prognosis in this series of patients with meningeal tuberculosis. Six of 8 patients with this form of extrameningeal tuberculosis were dead within twelve months following the onset of meningitis.

The relationship of active extrameningeal tuberculosis to survival in the 46 patients studied is shown in table 5.

Precipitating factors: In searching for a precipitating factor in regard to onset of meningitis in this group, no definite correlation with any causal agent was found. However, 6 patients (13 per cent) developed meningitis within one to four weeks following these surgical procedures: appendectomy, craniotomy, spinal fusion, ileocolectomy, epididymectomy, and orchiectomy. Another 2 patients developed meningitis coincident with pregnancy.

First appearance of meningitis during streptomycin-PAS therapy: Approximately one-fourth of these 46 patients (11 patients) developed clinically recognizable meningitis while receiving intermittent streptomycin and daily PAS for extrameningeal tuberculosis. On establishing the diagnosis of tuberculous meningitis, therapy was changed to daily parenteral streptomycin, and intrathecal streptomycin was withheld. Five of these patients died (45 per cent), and 6 survived. Four of the latter have reached the point of normal cerebrospinal fluid and are expected to recover under prolonged treatment. It is interesting to speculate whether or not the prompt addition of isoniazid in large doses to these 11 patients would have favorably influenced survival and decreased the time necessary for conversion of the cerebrospinal fluid to normal.

Emergence of drug-resistant tubercle bacilli: In only one patient in this series was there clear-cut evidence of the emergence of bacillary resistance in the cerebrospinal fluid while under treatment.

This occurred in a 32-year-old Negro veteran, who died of his disease. Prior to admission to this hospital he had received streptomycin alone for five months (70 gm.) for genitourinary tuberculosis and an acute hematogenous dissemination. On admission to Fitzsimons Army Hospital, he had a second hematogenous dissemination. The cerebrospinal fluid examined at that time was normal, and he was placed on alternating four-week courses of streptomycin and PAS, as previously described. Two months later meningitis developed and cerebrospinal fluid culture yielded *M. tuberculosis* susceptible to both streptomycin and PAS. Two months later the organisms recovered from the cerebrospinal fluid were highly resistant to both streptomycin and PAS[4]. The patient had received a total of 42 gm. of streptomycin and 1,040 gm. of PAS at this hospital and, following the appearance of meningitis, had maintained a cerebrospinal fluid streptomycin concentration between 20 to 50 γ per ml. on parenteral therapy. One month later the patient developed a complete subarachnoid block of the lumbar cord and died.

Post-treatment relapse: Relapse following discontinuation of treatment was observed in only one patient.

A 24-year-old, white male was admitted in 1948 with miliary and meningeal tuberculosis. For the next sixteen months he received alternating four-week courses of daily streptomycin or PAS. At that time his cerebrospinal fluid had been normal in all respects for three months. He remained asymptomatic for two months following the withdrawal of drug therapy, and then developed a clinical relapse. Repeated cultures of the cerebrospinal fluid at that time yielded *M. tuberculosis* susceptible to both streptomycin and PAS. These two drugs were again resumed and continued on the alternating regimen for the next twenty months. The cerebrospinal fluid again became normal within eight months, and chemotherapy was prolonged for twelve additional months. This patient

[4] As outlined in previous publication from this hospital (8), cultures showing growth on the 10 γ per ml. tube equal to that on the control (0 γ per ml.) were called resistant to the drug tested.

is now well and working part time, two years following completion of the second course of treatment.

Subarachnoid block: The development of clinically recognized block within the subarachnoid space occurred in 6 of the 19 treatment failures (33.7 per cent). Two of these patients had received intrathecal streptomycin for three and four weeks, respectively, and following this survived for two months and eleven months, respectively, before the development of a mechanical subarachnoid block resulted in death. The 4 patients not treated with intrathecal streptomycin survived for periods ranging from four weeks to nine months before this fatal complication appeared.

Two additional patients in this series developed a mechanical subarachnoid block which apparently resolved spontaneously on conservative management.

One patient, a 17-year-old housewife, had received one month of intrathecal streptomycin at another hospital and within two months had developed a complete block somewhere between the cisterna magna and the lumbar cord. Her cerebrospinal fluid protein level rose steadily to a level of 5 gm. per 100 ml. On conservative management with parenteral streptomycin, oral PAS, and isoniazid in large doses over the next two months, her general status improved remarkably and manometric studies revealed normal cerebrospinal fluid dynamics. The cerebrospinal fluid protein fell to the vicinity of 0.2 to 0.3 gm. per 100 ml., and treatment is being continued.

One additional patient also had clearing of a mechanical subarachnoid block on prolonged conservative management with triple drug therapy.

It was believed that there was no significant correlation between the route of administration or duration of streptomycin therapy and the development of subarachnoid block in this series.

DISCUSSION

Following the early widespread use of streptomycin in treating meningeal tuberculosis, many favorable reports appeared in the world literature. A preliminary report from the British Medical Research Council demonstrated the superiority of combined intrathecal and parenteral streptomycin over parenteral streptomycin alone in their case material (9). Other investigators began using intrathecal streptomycin in larger dosage and for more prolonged periods and reported results superior to those previously obtained with parenteral streptomycin (10). In an effort to raise the survival rate, catheter instillation of streptomycin to the inflammatory exudate in the subarachnoid space at the base of the brain was employed by this group. The major attack at this time was the administration of maximal concentration of drug to the organisms in the subarachnoid space before the host response to infection prevented proper diffusion and bacteriostasis of parenterally and topically applied streptomycin.

Most investigators are in agreement that, in the presence of inflammation in and about the meninges, an increased quantity of streptomycin appears in the cerebrospinal fluid when compared to the normal control values.

It has been shown that the quantity of streptomycin in the cerebrospinal fluid

fluctuates in direct proportion to the severity and duration of the meningeal inflammation (10, 11). As the inflammatory reaction subsides, the amount of parenterally administered streptomycin detectable in the cerebrospinal fluid diminishes, only to rise again should an inflammatory relapse occur. A rise in the streptomycin concentration of cerebrospinal fluid has been noted by others to indicate an impending relapse and to constitute an indication for resumption of intrathecal drug administration (10, 11).

In the present series of patients, analysis of data regarding cerebrospinal fluid concentrations of streptomycin has failed to reveal any consistent information that might aid in prognosis. Serial drug determinations in the cerebrospinal fluid of survivors were found to closely parallel the values obtained in patients who died (12). Survival appeared to have little, if any, relation to the concentration of streptomycin appearing in the cerebrospinal fluid. Several patients died with drug concentrations consistently between 15 and 30 γ per ml. of cerebrospinal fluid, and without clinical evidence of block. The one consistent finding in the surviving patients was the uniform lowering of the streptomycin concentration to the range of 1 to 5 γ per ml. coincident with the return of the cerebrospinal fluid to normal. Data on viomycin and PAS concentrations in the cerebrospinal fluid were found to closely parallel those noted with streptomycin.

It appears that, with present-day management of meningeal tuberculosis, approximately one-half of those treated can be expected to survive (7, 13, 14). The exact figures will vary considerably depending on the ages of the patients and the length of follow-up. In the present study it has been demonstrated that a survival rate of approximately 50 per cent can be obtained by prolonged conservative management without the use of intrathecal drugs. Furthermore, the data suggest that survival may be closely related to the extent of extrameningeal tuberculosis present and to the individual host response to the meningeal infection. These factors appeared to influence mortality as much as did the type of regimen employed or the drug concentrations achieved in the cerebrospinal fluid.

The pathogenesis of tuberculous meningitis has been adequately described by several writers (15, 16). It has been established that the unusual source of infection is the rupture of a caseous focus, either a cortical tubercle or a meningeal plaque, directly into the subarachnoid space of a sensitized subject. The offending lesion is the residual of a previous hematogenous dissemination. The relatively minor role of the blood-borne bacilli of a hematogenous dissemination in producing the picture of acute meningeal tuberculosis in the adult has also been emphasized by pathologists. The sudden flooding of the subarachnoid space of a sensitized individual by hosts of tubercle bacilli, and the necrotic debris from a ruptured cortical focus, evokes a powerful inflammatory reaction. Many of the early symptoms of meningeal tuberculosis can be explained on the basis of local tissue hypersensitivity acting within the rigid confines of the cranial vault. Under the protection of potent chemotherapeutic agents, the host may survive long enough to allow evolution of the acute inflammatory exudate into a chronic

inflammatory reaction which may interfere with the antimicrobial activity of drugs.

In an effort to change the poor prognosis of the chronic, organizing inflammatory reaction in certain patients with meningeal tuberculosis, the precipitation of an acute hypersensitivity reaction within the subarachnoid space has been accomplished (7). Intrathecal tuberculin combined with streptomycin has been used to evoke an intense inflammatory and fibrinolytic reaction at the site of the lesions, thus exposing protected tubercle bacilli to the activity of potent drugs. The potential hazards of this form of treatment have limited its widespread adoption in this country. Historically, however, it marks one of the first successful attempts to alter a deleterious host reaction to infection at the same time that vigorous measures are being taken against the infection agent. Proteolytic enzymes such as streptokinase and streptodornase have also been used intrathecally in an effort to alter the host response to infection (17). More recently the anti-inflammatory and mesenchymal inhibitory actions of the adrenal cortical hormones have been used in combination with streptomycin to treat tuberculous meningitis (18).

As more potent antibacterial agents are developed, there will be an increasing opportunity to destroy the offending organisms in and about the inflamed meninges before irreversible host responses prevent this action. From the data presented in this study, it is evident that continued progress in reducing the mortality of meningeal tuberculosis has coincided with the development and use of new drugs. Isoniazid combined with streptomycin and PAS will undoubtedly further increase the incidence of survival in this disease. But it is also apparent that progress in the development and use of antibacterial drugs has for the time being overshadowed the investigation and understanding of the factors governing the host response to this infection. It is not unlikely that some day the absolute limits of what can be expected by tuberculocidal and tuberculostatic drugs in meningeal tuberculosis will be reached. To progress further will require additional painstaking study of the factors governing tissue response to this infection and the development of methods of control.

SUMMARY

The results of a five-year study at Fitzsimons Army Hospital of 46 adult patients with meningeal tuberculosis have been presented. During this period 19 patients (41 per cent) have died, and 10 patients (21 per cent) have been discharged as recovered. Of the 46 patients, 36 have been treated without intrathecal streptomycin. Fourteen (38 per cent) of the latter have died, while 17 (47 per cent) have regained a normal cerebrospinal fluid. The 5 remaining patients, to date, are responding satisfactorily to treatment.

The changing concepts of the chemotherapy of tuberculous meningitis during this period have been discussed.

The results achieved to date with parenteral streptomycin, oral isoniazid, and para-aminosalicylic acid (PAS) suggest that this regimen offers the best hope for control of meningeal tuberculosis.

The interrelations of pathogenesis, host response to infection, and bacterial control by chemotherapy have been outlined.

SUMARIO

El Tratamiento de la Meningitis Tuberculosa: Estudio de Cuarenta y Seis Enfermos Adultos

Preséntase el resultado de un estudio de cinco años de 46 enfermos adultos con tuberculosis meníngea en el Hospital Militar Fitzsimons. Durante ese período, 19 (41 por ciento) enfermos han muerto y se ha dado de alta a 10 (21 por ciento) como curados. Del grupo, 36 fueron tratados sin estreptomicina por vía intratecal. Catorce (38 por ciento) de éstos han muerto, en tanto que 17 (47 por ciento) muestran de nuevo un líquido céfalorraquídeo normal. Los 5 sujetos restantes van respondiendo, hasta la fecha, satisfactoriamente al tratamiento.

Discútense los cambios que ha habido en nuestros conceptos de la quimioterapia de la meningitis tuberculosa durante el período en cuestión.

Los resultados conseguidos hasta ahora con la estreptomicina por vía parentérica, la isoniacida por vía oral y el ácido para-aminosalicílico (PAS) sugieren que ese régimen es el que ofrece mayores esperanzas para el dominio de la tuberculosis meníngea.

Bosquéjanse las interrelaciones de la patogenia, la mejor respuesta a la infección y el dominio bacteriano por la quimioterapia.

RESUME

Le traitement de la méningite tuberculeuse: Etude chez quarante-six adultes

Présentation des résultats d'une étude effectuée pendant cinq ans à l'Hôpital militaire Fitzsimons dans 46 cas de méningite tuberculeuse chez l'adulte. Au cours de cette période, 19 malades (41%) sont morts et 10 (21%) sont sortis de l'hôpital après guérison. Parmi 46 malades, l'injection sous-arachnoïdienne de streptomycine a été omise chez 36 d'entre eux. Quatorze (38%) membres de ce dernier groupe sont morts, alors que chez 17 (47%) le liquide céphalo-achidien est redevenu normal. A ce jour, les 5 autres malades réagissent favorablement au traitement.

Les différentes conceptions émises au sujet de la chimiothérapie de la méningite tuberculeuse au cours de cette période sont étudiées.

Les résultats acquis actuellement avec la streptomycine parentérale, l'isoniazide orale et l'acide para-aminosalicylique (PAS) suggèrent que cette thérapeutique permet les meilleurs espoirs quant au contrôle de la tuberculose méningée.

Les corrélations de la pathogénèse, réponse de l'hôte à l'infection et contrôle des bactéries par la chimiothérapie ont été esquissées.

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RESPONSE TO BCG VACCINATION AS AN INDEX OF FAMILIAL SUSCEPTIBILITY TO TUBERCULOSIS[1]

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INTRODUCTION

The tendency of tuberculosis to run in families may be largely attributed to epidemiologic factors: first and foremost, to the increased risk of infection when a member of a family first contracts the disease from the outside world. Still an open question, however, is the possibility of familial variation in susceptibility or resistance to tuberculosis. Lack of definite evidence in this domain is primarily the result of practical difficulties. To develop a suitable method for discriminating between constitutional and epidemiologic factors which operate in ordinary human tuberculosis would seem to involve almost insurmountable difficulties.

In recent studies (1, 2, 3), the problem has been attacked indirectly from quite a different angle. Instead of dealing with natural tuberculous infection and the influence of constitutional factors on its development and course, the measurable effects of a benign artificial tuberculous infection (BCG) have been investigated. Through the use of a statistical procedure known as the analysis of variance, it was found that the tuberculin reactions, as well as the local lesions, after BCG vaccination were more uniform in size among children belonging to the *same* family than in children from *different* families. In other words, a child's capacity to respond to artificially introduced tubercle bacilli is influenced by his familial constitution. Children of some families have the capacity to develop strong tuberculin allergy after vaccination, those of other families develop weaker allergy; and the same holds true for the capacity to develop local lesions at the vaccination site.

Assuming that the responses to a natural tuberculous infection depend also on constitutional factors, it could be questioned whether a person's capacity to develop allergy or vaccination lesions is somehow linked with his resistance to tuberculous disease. The present study was undertaken in an attempt to answer this question.

MATERIALS AND METHODS

The present study, together with several others, was a by-product of a large-scale investigation of BCG vaccine undertaken jointly by the International Tuberculosis Campaign, the State Serum Institute in Copenhagen, and the Tuberculosis Research Office of the World Health Organization (4). The investigation was carried out in a rural area of Denmark, and the subjects were schoolchildren, essentially all within the age span of seven to fourteen years, with an equal proportion of boys and girls. The previously unvaccinated children who gave less

[1] From the Tuberculosis Research Office, World Health Organization, Copenhagen, Denmark.

than 6 mm. of induration to an intradermal Mantoux test with 10 TU[2] were vaccinated in a series of studies dealing with different vaccines and vaccination techniques.

As an integral part of these studies, the size of the local vaccination lesion was measured three days and ten weeks after vaccination. Mantoux tests with 10 TU were also given after ten weeks (simultaneously with the second reading of the lesions) and again one year after vaccination, the transverse diameter of induration being recorded after an interval of seventy-two hours.

These recordings, obtained from children vaccinated in the first seven studies during the period from November, 1949, to October, 1950, provided one part of the basic material used in the present analysis. The other part consisted of specially collected information on the incidence of tuberculosis among the relatives of the children. The plan was then to combine the information obtained from the two sources in an attempt to determine whether the family factors known to influence the responses to BCG are related to some (hypothetical) family factors which may influence susceptibility (or resistance) to tuberculous disease.

As a first step in collecting data on incidence of tuberculosis in the families, one or both parents of the vaccinated children were interviewed. For practical reasons, the interviews had to be confined to a limited number of families, approximately 500, and the problem of selecting these families most efficiently from the total number available was therefore specially considered. As families with more vaccinated children provided more observations on the responses to vaccination, only those with at least two vaccinated children were included (too few families had more than two vaccinated children to set the limit higher). Further, significant differences in the incidence of tuberculosis would most probably be found between families differing widely with respect to the responses recorded in the vaccinated children. Both measures of response—size of local vaccination lesions as well as size of Mantoux reactions—might potentially be related to the degree of resistance. Consequently, the selection was made from a correlation diagram in which all families were distributed by mean size of the children's local lesions and Mantoux reactions, both recorded ten weeks after vaccination. Because vaccines of different strengths were used, the measured size of each reaction was converted to the deviation from the mean value of the reactions of all children vaccinated with the same vaccine.

The distribution of the families with two or more children by the two criteria is shown in table 1. The areas from which families were selected are bounded by heavy lines in the diagram. In all, 499 families were selected from a zone following the outer limits of the distribution so that various combinations of small and large lesions with low and high allergy, as far as possible, were equally well represented. Another 51 families were selected from the center of the distribution to provide a control group of typical or average responses.

Of the 550 families selected, 60 had to be omitted after the interviewing because the information collected was insufficient or because the children in the family appeared to be adopted. The study is therefore based on 490 families with, in all, 1,101 children, an average of 2.25 children per family.

The interview scheme included information on the children's parents and grandparents and on all of the sisters and brothers of both parents. In the following presentation they are referred to as the relatives of the children. From the interview the total number of relatives, dead as well as alive, was determined for each family; and, for those no longer

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[2] 1 TU (tuberculin unit): 1/50,000 mg. ref. standard purified protein derivative (PPD) or 0.01 mg. of international Old Tuberculin (OT) (0.1 cc. of 1/10,000 dilution).

TABLE 1

FAMILIES WITH TWO OR MORE BCG-VACCINATED CHILDREN DISTRIBUTED BY MEAN SIZE OF THE CHILDREN'S VACCINATION LESIONS AND MANTOUX REACTIONS TO 10 TU TEN WEEKS AFTER VACCINATION

VACCINATION LESION AFTER TEN WEEKS MEASURED FROM MEAN OF VACCINE GROUP	MANTOUX REACTION AFTER TEN WEEKS MEASURED FROM MEAN OF VACCINE GROUP (MM.)																						
	−6.0	−5.0	−4.0	−3.5	−3.0	−2.5	−2.0	−1.5	−1.0	−0.5	0	+0.5	+1.0	+1.5	+2.0	+2.5	+3.0	+3.5	+4.0	+5.0	+6.0		
mm.																							
−6.0			1						1														
−5.0						1					1												
−4.0								1	1	1	2					1	1				1		
−3.5	1	1			3	2	2	3	1	1					2		1						
−3.0	1	1	1	1	4	3	2	2	3		4	1	1		1	2	1						
−2.5		1	6	5		7	3	9	3	3	7	7	3	6	2	3	5		1	1	1	1	
−2.0	1	1	2	4	2	1	6	4	8	10	5	10	7	9	3	3	3	1	2	3	1		
−1.5	1	2	6	4	8	10	12	10	7	14	8	13	10	17	10	9	5	2					
−1.0	1	7	3	8	6	12	11	11	13	19	21	21	13	16	7	8	8	2	2	4		3	
−0.5	3	4	4	7	2	13	11	19	19	28	20	17	19	15	14	10	11	6	1	3	3	1	
0		1	7	9	11	5	13	14	14	19	15	19	24	11	15	10	10	7	7	4	3	2	
+0.5	1	1	7	4	7	13	7	12	13	19	18	24	18	13	18	13	5	7	7	7	3	3	
+1.0		1	2	2	2	9	6	13	9	11	8	21	12	9	14	13	5	7	3	9	3	3	
+1.5		2		1	1	6	6	9	10	15	5	11	17	5	13	10	5	6	4	7	4		
+2.0	1		2			4	1	7	4	10	5	6	14	7	7	3	5	4	6	4	3	2	
+2.5		2		3		1	1	6	1	3	5	11	8	5	8	2	6	6	2	5	2	1	
+3.0					2	2		1	1	6	2	12	5	7	4	5	4	4	2	7	3		
+3.5				1				3			2	3	3	1	6	3	4		1				
+4.0				1	3	1	2			1		3		2	5	2		2		2		2	
+5.0			1						2	2	4	4	3	3	3	2	4	1	4	1			
+6.0								1	1	4	2			3		1		1		1	1		
	1			1				2	1	1	2	2	1	3			1	1	1	2		1	

alive, the name and address at the time of death and the years of birth and of death were recorded. Whenever possible, the cause of death was entered on the interview forms, filled in by a statistically trained nurse. Tuberculous disease not resulting in death was not included in the analysis, as the definition and diagnosis of such cases would involve too much uncertainty.

As not too much confidence could be placed in the information obtained from the interviewed persons on the cause of a relative's death, great efforts were made to verify or supplement the information. All deaths after 1925 were compared with the tuberculosis card-index in the Danish State Health Department (Sundhedsstyrelsen). This card-index is based on official death certificates and contains information on all persons who have died from tuberculosis since 1925. Moreover, all deaths suspected to be due to tuberculosis and not found in the card-index, primarily because they occurred before 1925, were compared with the original death certificates filed in different archives in Denmark. The remaining unverified cases were compared, whenever possible, with the clinical records in hospitals and sanatoriums.

In tables 2 through 8, deaths among the children's relatives are classified into two groups: *verified tuberculosis deaths* and *possible tuberculosis deaths*. Verified tuberculosis deaths

TABLE 2

TUBERCULOSIS DEATHS PER 1,000 RELATIVES OF BCG-VACCINATED CHILDREN ACCORDING TO SIZE OF THE CHILD'S MANTOUX REACTION TO 10 TU TEN WEEKS AFTER VACCINATION

MANTOUX REACTION AFTER TEN WEEKS MEASURED FROM MEAN OF VACCINE GROUP	NUMBER OF CHILDREN	NUMBER OF RELATIVES	TUBERCULOSIS DEATHS PER 1,000 RELATIVES	
			Verified	Verified plus Possible
mm.				
– to −5.01	107	1,858	11.8	17.8
−5.00 to −3.01	108	1,953	16.4	22.0
−3.00 to −2.01	89	1,586	11.3	15.8
−2.00 to −1.01	100	1,773	9.6	13.5
−1.00 to −0.01	107	2,000	12.0	17.0
0 to +0.99	123	2,300	8.7	13.5
+1.00 to +1.99	88	1,657	10.9	13.9
+2.00 to +2.99	80	1,472	10.2	17.0
+3.00 to +4.99	151	2,787	11.5	12.9
+5.00 to	140	2,528	11.5	15.8
Not examined	8	149	20.1	26.8
Total	1,101	20,063	11.5	15.9

include only those in which the card-index, the death certificate, or hospital record explicitly indicated that the death was the result of tuberculous disease. Whenever doubt existed about the cause of death, the cases were listed as possible tuberculosis deaths. This heading also includes deaths reported in the interview as tuberculous but of which no record could be found in the archives.

RESULTS

For analysis of the results, the individual child has been considered the unit, which means that a particular relative appears as many times in the tables as there are children in the family. The same holds for the deaths from tuberculosis among the relatives. The reason for this is primarily the fact that the families in the study are of different sizes.

Among the total of 20,063 relatives thus counted, 230 verified and 88 possible deaths due to tuberculosis were found. The average number of verified tuberculosis deaths per 1,000 relatives was 11.5, and the average number of verified plus possible tuberculosis deaths was 15.9.

Table 2 and figure I show how the death rates vary with the size of the child's induration to the Mantoux 10 TU test ten weeks after vaccination. Verified tuberculosis deaths vary from 8.7 to 16.4 per 1,000 relatives, but no systematic pattern is found; verified deaths plus possible deaths vary from 12.9 to 22.0. There are slight indications of a higher mortality among relatives of children with low allergy, but the differences in the rates of tuberculosis deaths are not statistically significant. Testing the hypothesis of even distributions, the proba-

TABLE 3

TUBERCULOSIS DEATHS PER 1,000 RELATIVES OF BCG-VACCINATED CHILDREN ACCORDING TO SIZE OF THE CHILD'S MANTOUX REACTION TO 10 TU ONE YEAR AFTER VACCINATION

MANTOUX REACTION AFTER ONE YEAR MEASURED FROM MEAN OF VACCINE GROUP	NUMBER OF CHILDREN	NUMBER OF RELATIVES	TUBERCULOSIS DEATHS PER 1,000 RELATIVES	
			Verified	Verified plus Possible
mm.				
- to −5.01	64	1,198	16.7	21.7
−5.00 to −3.01	75	1,391	10.8	15.1
−3.00 to −2.01	57	1,083	11.1	12.9
−2.00 to −1.01	85	1,496	10.0	14.7
−1.00 to −0.01	100	1,795	12.3	15.0
0 to +0.99	99	1,747	9.7	14.3
+1.00 to +1.99	85	1,598	9.4	13.1
+2.00 to +2.99	64	1,117	15.2	18.8
+3.00 to +4.99	103	1,891	13.7	21.7
+5.00 to	66	1,107	11.7	17.2
Not examined	303	5,640	10.3	14.4
Total	1,101	20,063	11.5	15.9

TABLE 4

TUBERCULOSIS DEATHS PER 1,000 RELATIVES OF BCG-VACCINATED CHILDREN ACCORDING TO SIZE OF THE CHILD'S VACCINATION LESION THREE DAYS AFTER VACCINATION

VACCINATION LESION AFTER THREE DAYS MEASURED FROM MEAN OF VACCINE GROUP	NUMBER OF CHILDREN	NUMBER OF RELATIVES	TUBERCULOSIS DEATHS PER 1,000 RELATIVES	
			Verified	Verified plus Possible
mm.				
- to −1.51	112	2,079	13.5	18.8
−1.50 to −0.51	314	5,789	11.4	15.0
−0.50 to −0.01	210	3,710	8.6	14.0
0 to +0.49	161	2,823	14.5	18.8
+0.50 to +1.49	179	3,294	11.8	17.3
+1.50 to +2.99	81	1,456	8.9	11.0
+3.00 to	30	592	11.8	13.5
Not examined	14	320	12.5	18.8
Total	1,101	20,063	11.5	15.9

bilities found in chi-square tests were 0.70 for verified tuberculosis deaths and 0.50 for verified plus possible deaths.

The corresponding variation in tuberculosis death rates among relatives according to the children's Mantoux reactions after one year is given in table 3 and figure II. The relatives of children with reactions of intermediate size have a

TABLE 5

TUBERCULOSIS DEATHS PER 1,000 RELATIVES OF BCG-VACCINATED CHILDREN ACCORDING TO SIZE OF THE CHILD'S VACCINATION LESION TEN WEEKS AFTER VACCINATION

VACCINATION LESION AFTER TEN WEEKS MEASURED FROM MEAN OF VACCINE GROUP	NUMBER OF CHILDREN	NUMBER OF RELATIVES	TUBERCULOSIS DEATHS PER 1,000 RELATIVES	
			Verified	Verified plus Possible
mm.				
- to −5.01	27	501	16.0	24.0
−5.00 to −3.01	131	2,336	6.8	10.7
−3.00 to −1.51	191	3,532	8.5	13.9
−1.50 to −0.51	172	3,157	11.7	14.9
−0.50 to −0.01	74	1,401	17.8	23.6
0 to +0.49	49	898	11.1	15.6
+0.50 to +1.49	110	2,021	12.4	17.3
+1.50 to +2.99	116	2,063	12.1	17.5
+3.00 to +4.99	112	2,033	14.8	18.2
+5.00 to	115	2,043	11.3	14.2
Not examined	4	78	12.8	12.8
Total	1,101	20,063	11.5	15.9

TABLE 6

TUBERCULOSIS DEATHS PER 1,000 RELATIVES OF BCG-VACCINATED CHILDREN ACCORDING TO SIZE OF THE CHILD'S VACCINATION LESION AND MANTOUX REACTION TO 10 TU TEN WEEKS AFTER VACCINATION

TUBERCULOSIS DEATHS	VACCINATION LESION AFTER TEN WEEKS MEASURED FROM MEAN OF VACCINE GROUP (mm)	MANTOUX REACTION AFTER TEN WEEKS MEASURED FROM MEAN OF VACCINE GROUP					
		- −3.01	−3.00 to −1.01	−1.00 to +0.99	+1.00 to +2.99	+3.00- to	Total
Verified	- to −3.01	11.9	5.9	13.0	2.7	3.2	8.5
Verified plus possible		18.3	10.3	18.2	2.7	6.5	13.0
Verified	−3.00 to −0.51	12.9	11.2	9.1	8.0	8.6	10.0
Verified plus possible		17.0	15.9	13.6	13.8	11.9	14.4
Verified	−0.50 to +0.49	21.2	26.9	11.9	20.4	7.8	15.2
Verified plus possible		35.3	30.8	16.4	24.9	10.9	20.4
Verified	+0.50 to +2.99	18.6	5.7	10.2	9.1	13.6	12.2
Verified plus possible		27.1	7.6	15.8	15.2	17.2	17.4
Verified	+3.00 to	13.5	10.9	9.8	12.7	15.8	13.0
Verified plus possible		13.5	15.3	14.7	17.7	17.1	16.2
Verified	Total	14.2	10.4	10.2	10.5	11.5	11.5
Verified plus possible		19.9	14.6	15.1	15.3	14.3	15.9

TABLE 7

TUBERCULOSIS DEATHS PER 1,000 RELATIVES OF BCG-VACCINATED CHILDREN ACCORDING TO THE RELATION BETWEEN THE CHILD'S POST-VACCINATION ALLERGY AND SIZE OF VACCINATION LESION AFTER TEN WEEKS

POST-VACCINATION ALLERGY IN RELATION TO SIZE OF VACCINATION LESION AFTER TEN WEEKS	NUMBER OF CHILDREN	NUMBER OF RELATIVES	NUMBER OF VERIFIED TUBERCULOSIS DEATHS	VERIFIED TUBERCULOSIS DEATHS PER 1,000 RELATIVES	NUMBER OF VERIFIED PLUS POSSIBLE TUBERCULOSIS DEATHS	VERIFIED PLUS POSSIBLE TUBERCULOSIS DEATHS PER 1,000 RELATIVES
A. Low	372	6,630	85	12.82	118	17.80
B. Intermediate	279	5,137	64	12.46	86	16.74
C. High	442	8,147	78	9.57	110	13.50
Total	1,093	19,914	227	11.40	314	15.77
Chi-square test of significance			$P < 0.20$		$P < 0.10$	

TABLE 8

TUBERCULOSIS DEATHS PER 1,000 RELATIVES OF BCG-VACCINATED CHILDREN ACCORDING TO THE RELATION BETWEEN THE SIZE OF THE CHILD'S VACCINATION LESION AFTER TEN WEEKS AND THREE DAYS

SIZE OF VACCINATION LESION AFTER TEN WEEKS IN RELATION TO SIZE AFTER THREE DAYS	NUMBER OF CHILDREN	NUMBER OF RELATIVES	NUMBER OF VERIFIED TUBERCULOSIS DEATHS	VERIFIED TUBERCULOSIS DEATHS PER 1,000 RELATIVES	NUMBER OF VERIFIED PLUS POSSIBLE TUBERCULOSIS DEATHS	VERIFIED PLUS POSSIBLE TUBERCULOSIS DEATHS PER 1,000 RELATIVES
A. Small	423	7,725	76	9.84	106	13.72
B. Intermediate	257	4,609	48	10.41	72	15.62
C. Great	403	7,331	101	13.78	133	18.14
Total	1,083	19,665	225	11.44	311	15.81
Chi-square test of significance			$P < 0.10$		$P < 0.10$	

lower mortality than those of children who fall into the extreme groups, but again the deviation from an even distribution is not statistically significant. The probabilities found in chi-square tests as described above were 0.70 for verified deaths and 0.50 for verified plus possible deaths.

The data in the tables thus do not give any evidence of a relation between a child's capacity to develop tuberculin allergy and the tuberculosis mortality in his family.

Results from grouping the relatives according to the size of the children's vaccination lesions measured three days after vaccination are given in table 4 and figure III. Again, no significant relation may be demonstrated, the probabilities found in chi-square tests being 0.40 for both verified deaths and verified plus possible deaths.

The greatest deviation from an even distribution of the deaths from tubercu-

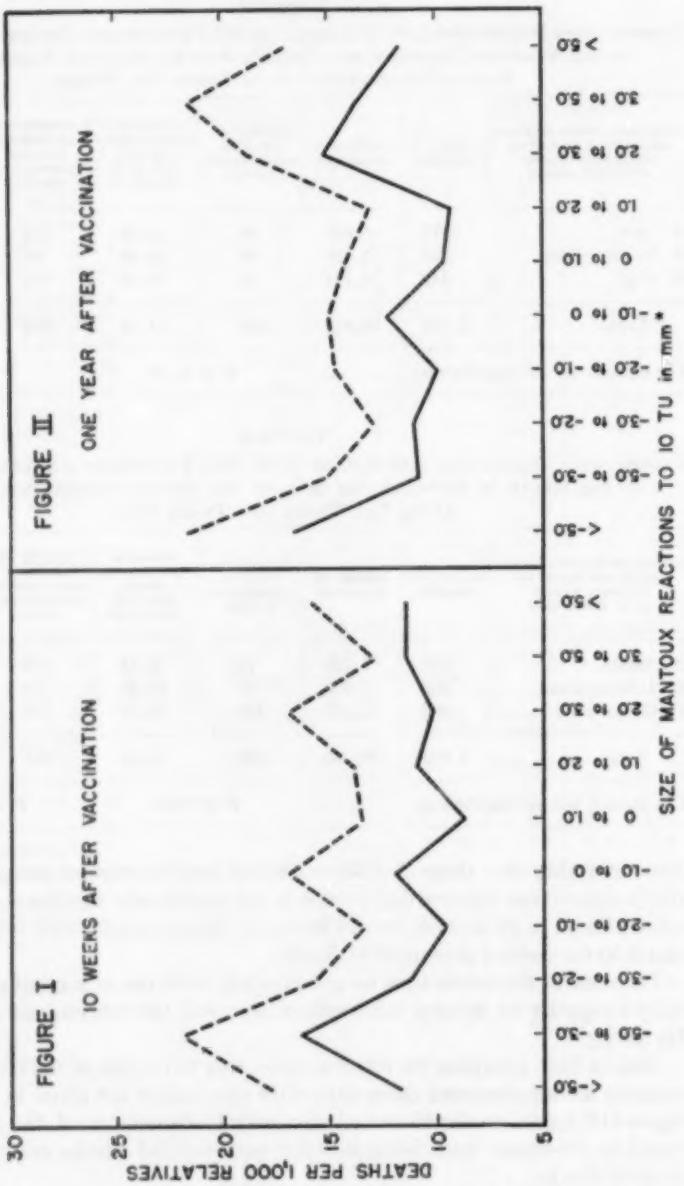
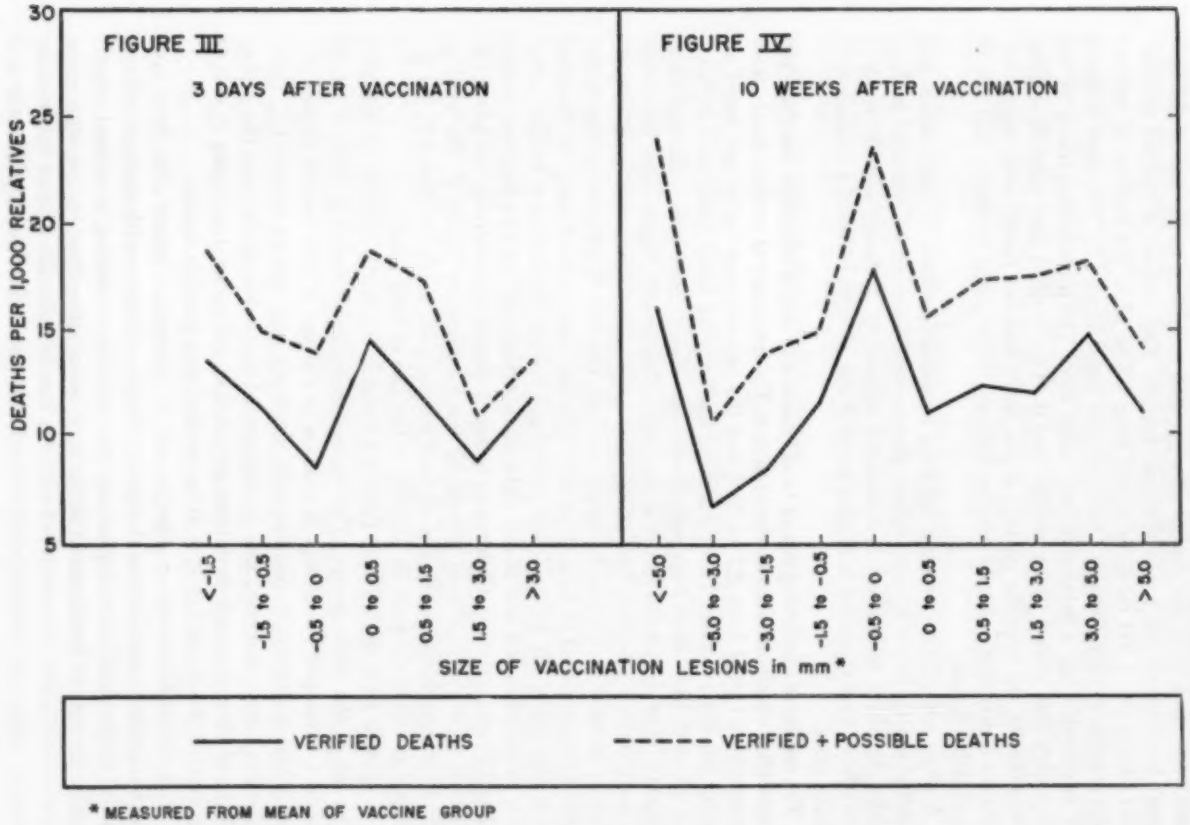


FIGURE I
10 WEEKS AFTER VACCINATION
FIGURE II
ONE YEAR AFTER VACCINATION
DEATHS PER 1,000 RELATIVES
30
25
20
15
10
5
<-30
-30 to -20
-20 to -10
-10 to 0
0 to 10
10 to 20
20 to 30
30 to 50
>50
SIZE OF MANTOUX REACTIONS TO 10 TU in mm*



FIGS. I–IV. Tuberculosis deaths per 1,000 relatives of BCG-vaccinated children by size of different indices of the child's response to vaccination.

losis was found when the deaths were grouped according to the size of the children's local lesions ten weeks after vaccination. The number of verified tuberculosis deaths per 1,000 relatives varied from 6.8 to 17.8; the number of verified plus possible deaths, from 10.7 to 23.6 (table 5 and figure IV). The curve in figure IV apparently has a maximum just below zero. The probabilities found by the chi-square test were 0.10 for verified and 0.20 for verified plus possible deaths. Nevertheless, the irregular pattern of the curve does not justify even vague conjectures concerning a simple relation between tuberculosis mortality and size of vaccination lesions.

A more complicated relation between measurable effects of vaccination and familial susceptibility to tuberculous disease may, however, be obscured in these tables, in which each effect is considered separately. Different combinations of two effects were therefore tabulated to see if any of them revealed a relation with death rates.

The results of combining size of local lesions and size of Mantoux reactions ten weeks after vaccination are shown in table 6. The frequency of verified deaths from tuberculosis varied from 2.7 to 26.9, but the two extreme values are based on rather few observations. There is a slight tendency for lower death rates in families whose children show high post-vaccination allergy associated with small local lesions (upper right-hand corner of the table). Conversely, higher rates are found in families with relatively low allergy and large lesions (lower left-hand corner). To test whether such a trend actually prevails, the data in adjacent groups in the table were combined to form three main groups: cases located along the diagonal from the upper left to the lower right-hand corners constitute a middle group (*B*); those below left and above right of this diagonal, the two extreme groups (*A* and *C*, respectively). Children in *Group A* developed relatively low allergy in relation to the size of local lesions; in the intermediate *Group B*, the level of allergy corresponded to the size of local lesions; and, finally, in *Group C*, the allergy was relatively great in relation to the size of local lesions.

It appears from table 7 that there is a trend in the death rates of the relatives who fall in the three groups. The highest mortality is found in families whose children developed low allergy in relation to the size of local lesions (*Group A*); the lowest mortality, in families with relatively high allergy (*Group C*). These differences may, however, also be interpreted as random deviations, the probabilities of obtaining such deviations as tested by chi-square tests being $P < 0.20$ for verified deaths and $P < 0.10$ for verified plus possible deaths.

Next, the children were grouped by size of vaccination lesions after three days and ten weeks to see whether changes in the size of lesions, with time, are related to the tuberculosis mortality among the relatives. According to animal experiments, the rate of development of the local lesion rather than its size after given time intervals may be connected with the innate resistance against tuberculous disease. Lurie and co-workers (5) found that the lesions developed faster and reached their maximum earlier in rabbits of genetically resistant strains than in those of susceptible strains. (It may be of special interest that this finding in

animals with *native* resistance resembles the Koch phenomenon observed in animals which have been *artificially immunized* against tubercle bacilli.)

The human counterpart of this finding would be a low incidence of tuberculosis in families whose children show a rapid development of vaccination lesions and a high incidence when the development is slow. As above, three main groups were formed from the table correlating size of vaccination lesions at three days and ten weeks: children with relatively large lesions after three days, and with relatively small ones after ten weeks, indicating rapid development of the lesion (*A*); those with the opposite combination, indicating slow development (*C*); and those in an intermediate group (*B*). The death rates per 1,000 relatives in each group have been computed and entered in table 8. The rates show a slight trend in the direction expected according to the animal results: lowest in *Group A*, highest in *Group C*. The deviation from a uniform frequency in all three groups is not statistically significant, however, as tested by a chi-square test ($P < 0.10$ for verified deaths as well as for verified plus possible deaths).

DISCUSSION

So far the present study has failed to indicate that different levels of tuberculin allergy or local lesions within the first year after vaccination reflect familial differences in the susceptibility or resistance to tuberculosis. There are indications that the combination of small local lesions and high allergy (both recorded ten weeks after vaccination) signifies greater familial resistance than the inverse combination and also that a rapid development of local lesions may be associated with resistance. None of the results, however, satisfies the usual requirements of statistical significance. In an attempt to explain the negative results, several possibilities might be considered.

One interpretation of the findings would be that the property of contracting disease as a result of tuberculous infection is independent of family factors. For general reasons this explanation does not seem plausible. The development and extent of tuberculous disease is a response to the invasion of tubercle bacilli. It seems unlikely that this type of response should not depend on factors similar to those which influence the measurable and benign effects of artificially introduced BCG.

Secondly, differences in susceptibility may be assumed to exist without being correlated with the ability to develop allergy or a local reaction at the vaccination site. Such a hypothesis would be compatible with the findings that allergy level and development of vaccination lesions have been shown to depend on certain familial factors, but it is also difficult on general grounds to believe that these familial characteristics should operate entirely independently of those governing the response to a virulent tuberculous infection. In animal studies, Lurie and associates (5) found that strains with different degrees of hereditary resistance against tuberculosis showed differences in the rate of development of vaccination lesions as well as differences in allergy-producing capacity. Tuberculin sensitivity developed faster and reached a higher maximum in genetically resistant than in susceptible strains.

It may be that the present material is too small and too homogeneous to disclose a corresponding correlation between family characteristics. Several possible sources of error can be pointed out. Measurements of tuberculin reaction and vaccination lesions represent the result of several steps: vaccination, the giving of tuberculin tests, and the recording of the reaction sizes (each of which is subject to experimental or observational errors). Again, the data collected in the interviews may be incomplete and the verification of deaths may be based to some extent on wrong diagnoses, particularly in regard to the older death certificates. Finally, it must be taken into account that the hereditary properties of the vaccinated child and the relatives included in the study, although all are descendents of the child's grandparents, may show considerable variation.

No systematic bias seems to be involved in any of the errors mentioned. Nevertheless, their total effect may be so great that considerably more material than the present may be required to show a relation, if any, between susceptibility to natural tuberculous infection in human beings and the measurable responses to BCG vaccination.

SUMMARY

Previous studies showed that familial factors influence the degree of tuberculin allergy and the size of the local lesions following BCG vaccination of schoolchildren. The object of the present study was to investigate the possibility of a relation between these factors and familial resistance or susceptibility to tuberculosis.

For this purpose, tuberculosis death rates among the relatives were determined for 1,101 BCG-vaccinated children belonging to 490 families These rates were then compared with the measurable indices of the children's responses to BCG vaccination.

Simple relations could not be demonstrated between the level of post-vaccination allergy or the size of the vaccination lesions in the children and tuberculosis mortality among their relatives. However, slightly lower death rates were found in families whose children showed a rapid development of vaccination lesions, compared with those who developed local lesions more slowly. While the differences are not statistically significant, they are in accordance with animal experiments, indicating that the rate of development of vaccination lesions may serve as an index of hereditary resistance to tuberculosis.

SUMARIO

La Respuesta a la Vacunación BCG como Indice de la Susceptibilidad Familiar a la Tuberculosis

Estudios anteriores han demostrado que factores familiares afectan la intensidad de la alergia a la tuberculina y el tamaño de las lesiones locales consecutivas a la vacunación con BCG de los niños de escuela. El objeto del estudio actual fué investigar la posible relación entre dichos factores y la resistencia o susceptibilidad familiar a la tuberculosis.

Para dicho fin, determináronse los coeficientes de mortalidad tuberculosa entre los parientes de 1,101 niños vacunados con BCG, que pertenecían a 490 familias distintas, y se compararon luego esos coeficientes con los índices mensurables de la respuesta de los niños a la vacunación con BCG.

No pudieron descubrirse relaciones simples entre la intensidad de la alergia postvacunal o el tamaño de las lesiones vacunales en los niños y la mortalidad tuberculosa entre sus parientes. No obstante, observáronse coeficientes ligeramente más bajos de mortalidad en las familias cuyos niños revelaban rápido desarrollo de lesiones vacunales, comparados con los que mostraban lesiones locales con más lentitud. Si bien las diferencias no son estadísticamente importantes, concuerdan con los experimentos en animales que indican que la velocidad de la aparición de las lesiones vacunales puede servir de índice de la resistencia hereditaria a la tuberculosis.

RESUME

La réponse à la vaccination par le BCG, comme indice d'une sensibilité familiale à la tuberculose

Des études antérieures ont montré que des facteurs familiaux influencent le degré de l'allergie à la tuberculose et l'importance des lésions locales de la vaccination par le BCG chez l'enfant d'âge scolaire. L'objet de l'étude actuelle était la recherche d'une relation possible entre ces facteurs et la résistance ou la sensibilité familiale à la tuberculose.

Dans ce but, le taux de mortalité par tuberculose parmi les parents a été déterminé chez 1.101 enfants vaccinés par le BCG et appartenant à 490 familles; ces taux furent comparés aux indices mesurables de la réaction vaccinale au BCG.

Il n'a pas été possible d'établir de rapports simples entre le degré de l'allergie postvaccinale ou l'importance des lésions vaccinales chez les enfants, et la mortalité par tuberculose parmi les membres de leur famille. Toutefois, un taux de mortalité légèrement plus faible a été enregistré dans les familles où les enfants présentaient une réaction vaccinale accélérée, par comparaison avec ceux chez lesquels le développement de lésions vaccinales locales était plus lent. Bien que ces différences ne soient pas significatives, du point de vue statistique, elles s'accordent avec l'expérimentation sur l'animal qui indique que la vitesse de développement des lésions vaccinales peut servir d'indice de résistance héréditaire à la tuberculose.

Acknowledgment

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AIR EMBOLISM COMPLICATING PNEUMOPERITONEUM[1]

A Review

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INTRODUCTION

Artificial pneumoperitoneum for the treatment of tuberculosis has become widely used in recent years (4). In some clinics, nearly every patient with active tuberculosis may expect to have pneumoperitoneum suggested at some time during the course of treatment. The chief reasons for the popularity of the procedure appear to be its wide applicability, the apparently simple and easy technique, and especially its relative freedom from serious complications. However, many ill effects from pneumoperitoneum have been witnessed. The most feared complication of pneumoperitoneum, as of pneumothorax, is the serious accident of air embolism. The frequency of fatal outcome, especially since it often occurs in an ambulatory patient on the road to recovery, has intensified this fear. With the increasing use of pneumoperitoneum, air embolism has occurred more frequently, as indicated by recent reports (*see* table 1 and bibliography).

VOLUME OF AIR NECESSARY FOR EMBOLISM

The amount of air which causes symptoms or fatalities varies chiefly with its site of entrance and localization. Many hundreds of centimeters may gradually enter a systemic vein and be absorbed or excreted in the lungs without ill effects. On the other hand, a minute amount of air may be fatal if it enters a pulmonary vein directly or enters a systemic vein and then travels across an inter-auricular or ventricular shunt to the left heart. The latter, known as paradoxical air embolism, can thus involve the cerebral or coronary arteries.

SITUATIONS WHICH MAY GIVE RISE TO AIR EMBOLISM

Air may enter into the circulation accidently in the course of obstetrical, gynecological, surgical, diagnostic, or therapeutic procedures. Accidental air embolism may also result from rapid decrease in intrapulmonary pressure as in caisson disease, deep-sea diving, or rapid airplane ascent. This also occurs from artificial positive pressure respiration with formation of air bubbles by release of dissolved nitrogen throughout the body. Simple atmospheric pressure may cause air to enter a vessel held open during trauma or surgery of the chest. Any positive pressure of air in a body cavity may enter into traumatically opened veins, especially pulmonary veins, during trauma to the chest, rib fracture, or lacerations. Air may also enter a peripheral vein, as result of pneumatic injection

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into the anus (criminal or accidental), skin, uterus in abortion, or the diagnostic Rubin test.

Air embolism has been encountered chiefly in the initiation or reinstillation of air in artificial pneumothorax from air entering a pulmonary vein, thus going directly to the left heart and not being filtered out in the lungs. Peripheral veins in the chest wall or pleural adhesions may similarly be entered. This also occurs without injection when air from the pleura or bronchi enters a torn or punctured vein which is under negative pressure but is kept from collapsing by infiltration or fibrosis. Extrapleural pneumothorax with high positive pressure increases this danger. Direct cavity puncture by exploring needle, as in McDowell or Monaldi procedures or cavernostomy, may also produce air embolism. Liver puncture may occur during right artificial pneumothorax, especially if the diaphragm is elevated in expiration, or with phrenic paralysis. In surgical cases, air embolism has also occurred during thoracoplasty, especially from intercostal veins held open along ribs by fibrosis. During pneumolysis, air may enter veins which are held open by adhesions.

Air embolism may occur in the course of artificial pneumoperitoneum from retroperitoneal emphysema which may dissect to the mediastinum and thence into the lung, peripheral subcutaneous emphysema, intravascular injection in abdominal wall, or puncture of spleen or liver. Liver puncture is by far the most common mechanism.

INCIDENCE OF AIR EMBOLISM IN PNEUMOPERITONEUM

The real incidence of air embolism in pneumoperitoneum is unknown. Only 100 case reports have been found in the literature. Nonfatal air embolism may be unsuspected, asymptomatic, or manifested by symptoms interpreted as mechanical, neurotic, reflex, or psychogenic, while even fatal air embolism, particularly if death is delayed and does not occur on the table, is apt to be ascribed to heart failure, anoxia, or other causes. More than a score of deaths from air emboli in the course of pneumoperitoneum treatment in California alone during the past few years and 11 cases recently reported from a single hospital in Uruguay (51) indicate that this condition is by no means as rare as is usually assumed.

Among 1,800 patients receiving artificial pneumoperitoneum at Olive View Sanatorium who had more than 80,000 refills between 1920 and 1952, 2 had fatal air embolism which was confirmed at autopsy. One other death resulted from inadvertent liver puncture during an attempted right pneumothorax. In addition, 3 instances of nonfatal air embolism were seen. One had repeated episodes with splenic punctures, which were revealed at autopsy when the patient died of air embolism during an attempted initial pneumothorax after the pneumoperitoneum was abandoned. The other 2 patients survived liver puncture complicating a right pneumoperitoneum. Additional cases of fatal air embolism following pneumoperitoneum have been described in personal communications from other places. The salient features of 100 cases reported in the literature, plus 20 previ-

ously unreported cases, and of the 7 cases observed here at Olive View are presented in table 1.

RELATION TO SITE OF ADMINISTRATION OF PNEUMOPERITONEUM

Although pneumoperitoneum is most often given in the left abdomen below the umbilicus, it is sometimes given below the ribs on either side. The spleen is rarely punctured, although this has occurred, but liver puncture is much more likely. The site of injection of air in most cases of air embolism was in the right hypochondrium. Three of 5 of the fatal cases at Olive View were proved at autopsy to have resulted from air injections directly into the liver, and 2 others were presumably due to liver puncture. One case showed splenic punctures, and

TABLE 1

DATA BASED ON REVIEW OF 100 CASES OF AIR EMBOLISM FOLLOWING PNEUMOPERITONEUM REPORTED IN THE LITERATURE PLUS 2 PREVIOUSLY UNREPORTED CASES AND 7 CASES FROM OLIVE VIEW SANATORIUM

Year Reported

YEAR	NUMBER OF REPORTS	NUMBER OF CASES		
		Nonfatal	Fatal	Total
1920	3	1	3	4
1930	7	7	2	9
1940	24	10	21	31
1950	28	30	26	56
Unpublished communications	15	2	18	20
Olive View series	1	3	4	7
Total	78	53	74	127

Administration

INDICATION	NUMBER OF CASES		
	Nonfatal	Fatal	Total
Treatment (tuberculosis)	15	22	37
Diagnosis (nontuberculosis)	1	7	8
Initial instillation	6	9	15
Refill	9	16	25
Side			
Right	3	13	16
Left	5	6	11
Amount			
Less than 250 cc	4	9	13
250 to 499 cc	3	7	10
500 cc	4	3	7

Symptoms

INDICATION	NUMBER OF CASES		
	Nonfatal	Fatal	Total
Restlessness	4	5	9
Convulsions	3	7	10
Unconsciousness	10	9	19
Paralysis	8	2	10
Reflexes absent	2	2	4
Headache or dizzy	4	2	6
Pupils dilated	4	4	8
Involuntary or incontinent	4	2	6
Blindness	3	0	3
Paresthesia face	1	0	1
Psychosis	1	0	1
Perspiration profuse	3	0	3
Pain in chest	3	3	6
Pulse absent	1	4	5
weak	9	6	15
Blood pressure fall	2	2	4
Dyspnea	5	8	13
Apnea	3	11	14
Cyanosis	6	12	18
Nausea and vomiting	1	1	2
"Collapse" or "syncope"	3	3	6
Rales, wheezing, or bubbling in chest	3	1	4
Froth at mouth	2	1	3
"Mill wheel" murmur	1	1	2
Distended neck veins	1	0	1
Pallor or flushed face	0	2	2
Observations			
Duration			
Less than 1 hour	2	17	19
1 to 23 hours	8	3	11
24 hours or more	7	3	10
Electrocardiograms obtained	4	1	5
"Normal"	1	0	1
Right heart "strain"	3	1	4
Other abnormalities	1	0	1
Treatment			
Drugs	3	9	12
Oxygen	3	6	9
Position (left side or head down, or both)	4	2	6
Autopsy	1*	18	19
Air in heart: right	0	7	7
Air in heart: right and left	0	2	2
Air in heart: unspecified	0	1	1
Air in brain	0	3	3
Air elsewhere	0	4	4
Liver puncture	0	9	9
Spleen puncture	1	2	3

* Case came to autopsy after pneumoperitoneum was abandoned.

the 2 patients surviving air embolism followed air injection into the right hypochondrium, probably due to liver puncture.

The frequency of fatal embolisms from simple liver biopsy is low, but, when air is injected into the liver, particularly in large amounts, fatality is apt to occur. The presence of a patent foramen ovale intensifies this danger. Patent foramen ovale has been found in approximately one-fifth of all autopsies but was seen in 2 of 4 cases of fatal air embolism autopsied at Olive View Sanatorium. Only one of the Olive View cases followed initial pneumoperitoneum, none occurred on the second instillation, and 5 followed later refills. There were 2 females and 5 males, all more than thirty years of age. Air was found in the heart four times, in the brain twice, and in abdominal vessels once.

DIAGNOSIS

In most cases the diagnosis of air embolism becomes obvious with dramatic suddenness, usually while the patient is receiving pneumoperitoneum on the table or shortly thereafter. The symptoms vary with the site of injection, the amount of air entering the circulation, the position of the body, and the effectiveness of the pulmonary excretory mechanism. Abdominal pain is usually the patient's first symptom, especially if the liver or spleen has been punctured. Then in rapid succession the patient may complain of chest pressure, dyspnea, or suffocation. He may rapidly become cyanotic, have a convulsive seizure, lapse into unconsciousness, and stop breathing. The heart sounds at first are rapid, then weak and irregular, and finally cease to be heard. If the air rapidly gets into the arterial circulation, the patient may develop symptoms of arterial embolism. In one of the Olive View cases it was possible to trace the arterial embolism as the air lodged in the different organs gave rise to symptoms peculiar to the affected organ. When the retinal vessels are occluded, transient blindness may occur, and when embolization occurs in the brain, paralysis and hemiplegias are common. In cerebral embolism, respiratory arrest may occur if the medulla is affected. Pain in the abdomen or extremities often occurs when air travels to the arterial circulation of the lower half of the body.

Symptoms may be delayed if air is pocketed off at the site of injection and later breaks into the circulation. If small amounts begin to circulate in the blood stream, the symptoms may be vague or varied and often bizarre, simulating other conditions or complications. In nervous individuals, these symptoms are often attributed to psychoneurosis. In still others, they may simulate serious cardiac disease. Awareness of air embolism as a complication of pneumoperitoneum is important and usually leads to the true diagnosis. If feasible, an electrocardiogram may be helpful by revealing an acute right heart "strain" pattern or conduction changes with varying degrees of heart block. Response to treatment by placing the patient on his left side, with the head down, may help confirm the diagnosis. A "mill-wheel" murmur is often described and is due to the air in the heart mixing with the blood. Roentgenograms of the abdomen, heart, and brain may reveal air in these parts and thus establish the diagnosis. In extreme cases, cardiac aspiration may reveal air in the heart which, when relieved, gives sudden improvement.

MECHANISM OF CIRCULATORY INTERFERENCE BY AIR EMBOLISM

The entrance of air or other nonabsorbable gas into the blood stream, and its transportation or movement within the vascular system, may result in embolism or interference with circulation in several ways. Fibrin clots may form around the air similar to extravascular clotting. Increased internal resistance and viscosity may result from greater extent of the interfaces of the air bubbles, especially when churned into a foam. Greater elasticity may enable the bubbles, especially when large, to slip back through cardiac valves. Because of their greater surface tension, large bubbles may fail to break up and so offer greater resistance as they encounter bifurcations or narrowing of vessels. The air emboli may act as solids and lead to infarction, or may act only as temporary blockages if the gases resorb or pass on.

The stoppages of circulation may be benign if the gas is soon resorbed; insignificant, if small or compensated for by collateral circulation, especially if they occur in unimportant tissues. On the other hand, a large persistent air embolism in important organs may block circulation entirely and prove as fatal as in the heart—coronary occlusion or medullary infarction affecting vital organs.

From a cardiac standpoint, air embolism is always serious. An air trap may form in the right ventricular outflow tract, resulting in obstruction to the blood flow through the lungs. This may lead to backward cardiac failure with increased venous pressure and cyanosis, and/or forward cardiac failure with falling blood pressure, rapid thready pulse, arrhythmias, myocardial ischemia, and shock.

TREATMENT

As soon as symptoms occur, the patient should be treated for air embolism. Durant and associates (12) have shown experimentally how placing the animal on his left side relieves the intracardiac obstruction from pulmonary air embolism because the air trapped in the right ventricular outflow tract floats toward the apex of the right ventricle. The air in the right ventricle is then churned into small bubbles and is allowed to pass through the pulmonary vessels to be excreted by the lungs. However, such an effort may not prevent intracardiac thrombosis, so may not save the patient from a cardiac death. Placing the patient on the left side with head down is good treatment since the air is then shunted away from the vital areas and into the lower portions of the body, thus avoiding a fatal outcome.

Procaine amide (pronestyl) may be tried intravenously or butacaine may be instilled directly into pericardial sac since this reduces heart irritability and may offset a fatal ventricular fibrillation. Cardiac aspiration of air in the heart has been tried. Stallworth and associates (42) reported this successfully in one case. Heart massage in cardiac standstill has also been advocated, but sufficient experience with this procedure is still lacking. If heart massage is done, the patient should be placed on the left side in a high Trendelenberg position to avoid forcing air into the brain.

PREVENTION DURING PNEUMOPERITONEUM

Pneumoperitoneum is not entirely devoid of danger. Among its complications must be recognized air embolism, which is often fatal. This danger is greater at

the initial pneumoperitoneum. Pneumoperitoneum patients should be fluoroscoped prior to therapy just as pneumothorax patients. Air should always be injected on the left side below the umbilicus. If the right side is used, one should be extremely careful of a liver puncture. It is good practice to palpate both lobes of the liver before initial therapy. Although there are a few instances in which a right-sided pneumoperitoneum may be used because of left-sided abdominal adhesions, as a general rule the right side should be avoided. If the left side cannot be used, it may be better to abandon the pneumoperitoneum than risk a fatal air embolism. In the left-sided pneumoperitoneum, the abdomen should be palpated for splenomegaly prior to institution of therapy.

Awareness of air embolism is the key to diagnosis. Electrocardiograms taken on patients at the time of embolism may show a specific pattern of right heart "strain," the degree depending on the amount of air in the heart and the resultant dilatation and anoxemia of the cardiac muscle. Prompt treatment of air embolism by placing the patient in the left lateral position helps by relieving the air trap in the outflow tract of the right ventricle, and by keeping the head down air is shunted away from the vital centers in the brain.

SUMMARY

The literature on air embolism has recently been mounting, stressing the frequency of this complication. From the growing literature, one hundred cases have been reviewed and twenty-seven additional cases have been added. The frequency of air embolism with right-sided pneumoperitoneum is so high as to constitute a contraindication to the use of the right side in pneumoperitoneum. A characteristic electrocardiographic pattern of right heart "strain" may aid in the diagnosis of air embolism. Lowering the head and placing the patient in the left lateral position is recommended as the best position during treatment of this complication. The newer drugs such as procaine amide (pronestyl) and butacaine should be added to the emergency drugs available whenever air collapse therapy is used.Cardiac aspiration or massage should be tried if all other treatments fail to revive the patient.

SUMARIO

Embolia Gaseosa como Complicación del Neumoperitoneo

La literatura relativa a la embolia gaseosa ha ido creciendo recientemente, recalcando así la frecuencia de esta complicación. De esa literatura creciente, se han repasado 100 casos, agregándoles 27 más. La frecuencia de la embolia gaseosa en el neumoperitoneo del lado derecho es tan alta que constituye una contraindicación del empleo del lado derecho para dicho procedimiento. Un típico patrón electrocardiográfico de tensión en el corazón derecho puede ayudar en el diagnóstico de embolia gaseosa. Como mejor posición durante el tratamiento de esa complicación, recomiéndase bajar la cabeza y colocar al enfermo del lado izquierdo. A las drogas destinadas a casos de urgencia en la sala de "nuemos," hay que agregar las más recientes, como Pronestil y Butacaína. Si los demás tratamientos no reviven al enfermo, deben probarse la aspiración o el masaje del corazón.

RESUME

Pneumopéritoine compliqué d'embolie gazeuse

Le nombre des communications sur des cas d'embolie gazeuse s'est récemment élevé, ce qui met en évidence la fréquence de cette complication. Cent cas extraits de cette littérature

croissante, ainsi que vingt-sept cas additionnels, ont été examinés. La fréquence de l'embolie gazeuse associée au pneumopéritoine droit est si élevée qu'elle constitue une contre-indication à l'emploi du côté droit comme siège du pneumopéritoine. Un tracé électrocardiographique caractéristique de l'effort du coeur droit peut faciliter le diagnostic de l'embolie gazeuse. La meilleure position à prescrire pendant le traitement de cette complication est l'abaissement de la tête et la position latérale gauche du corps. Les drogues récentes, telles que le Pronestyl et la Butacaïne doivent être ajoutées à la médication d'urgence du service d'insufflation. L'aspiration ou le massage cardiaque doivent être tentés pour ranimer le malade lorsque les autres traitements échouent.

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STUDIES ON THE TUBERCULOINHIBITORY PROPERTIES OF ASCORBIC ACID DERIVATIVES AND THEIR POSSIBLE ROLE IN INHIBITION OF TUBERCLE BACILLI BY URINE[1,2]

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INTRODUCTION

The report that urine is inhibitory for tubercle bacilli has appeared in the literature from time to time (1–5). Bjornesjo (3–5) recently conducted extensive investigations on the tuberculoinhibitory properties of urine. He reported that the majority of urines of healthy and tuberculous humans possess tuberculostatic and tuberculocidal properties. The activity was noted to vary with different persons and with the same person at different times. The most active urine tested produced complete inhibition of growth of tubercle bacilli in culture medium containing concentrations of the urine as low as 12.5 per cent. Inasmuch as the activity did not correlate with tuberculin sensitivity in healthy subjects and was not elevated in tuberculous subjects, it did not appear to be related to the tuberculous process or to specific antibody. In his attempts to identify the tuberculoinhibitory substance in urine, Bjornesjo tested a great many known urinary constituents. None of them was tuberculoinhibitory in concentrations normally present in urine. He observed that the active principle is a nonvolatile, dialysable, negatively charged, heat-stable substance which is resistant to both acid and alkali. It was found to be soluble in water and ethanol, but insoluble in acetone, ether, petroleum ether, benzene, carbon tetrachloride, and chloroform.

It occurred to the present writers that the properties of the urinary substance of Bjornesjo are not unlike those of ascorbic acid and certain of its derivatives. The well-known fact that the ascorbic acid content of urine fluctuates markedly with dietary intake is consistent with the marked variability in the tuberculoinhibitory activity of urines noted by Bjornesjo. Moreover, the observation that the addition of ascorbic acid to culture medium renders it strongly tuberculostatic for human strains of tubercle bacilli (6, 7) lends tenability to the hypothesis that ascorbic acid may be responsible in some manner for the tuberculoinhibitory property of urine.

The idea that the ascorbic acid of urine and serum may exert tuberculostatic action is not new. Pichat and Minjat (7) and Pichat and Reveilleau (8) conducted

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studies on the tuberculostatic properties of the serums and urines from human subjects following the intravenous administration of 3 gm. of ascorbic acid buffered with sodium bicarbonate. These investigators observed that normal human serums have a certain low tuberculostatic activity which was not changed by the administered ascorbic acid. On the basis of the recent work of Myrvik and Weiser (9), it seems probable that the low tuberculostatic activity of serums noted was due to lysozyme. Pichat and co-workers also noted that the urines voided before the injection of ascorbic acid were apparently without tuberculostatic activity since growth occurred after fifteen days of incubation in broths containing 50 per cent of the urine. By contrast, the urines taken after the injection of ascorbic acid produced tuberculostasis in culture medium to which as little as 15 per cent of such urines was added. These observations are not inconsistent with the concept of the present writers that the tuberculostatic activity of urine reported by Bjornesjo in some way depends on ascorbic acid.

Ascorbic acid is notoriously unstable in oxidizing environments (10–12). The instability of dilute solutions at pH values below 7.0 is often due to the catalyzing action of traces of metals such as copper and iron, whereas at higher pH values the oxidation is spontaneous (13, 14). It has been reported that, in the first oxidative step, ascorbic acid is converted to dehydroascorbic acid, which then mutarotates to 2,3-diketogulonic acid (10, 11, 12). At pH values above 6.2, diketogulonic acid readily undergoes an oxidative split which results in the formation of oxalic acid and L-threonic acid (12). Small quantities of furfurol may also result from the oxidation of ascorbic acid, especially at very low pH values (15). Borate buffer at pH 7.4 protects diketogulonic acid against oxidation (12). Ascorbic acid can be rapidly oxidized to dehydroascorbic acid by the addition of oxidizing substances such as iodine and chlorine water or by treatment with charcoal (11). Although ascorbic acid readily undergoes oxidation under the many conditions mentioned, certain substances, especially of reducing nature, such as glutathione and hydrogen sulfide inhibit its oxidation (11). It is notable that dehydroascorbic acid may be readily reconverted to ascorbic acid with hydrogen sulfide, whereas diketogulonic acid cannot be reconverted to dehydroascorbic acid or to ascorbic acid by such treatment (11).

It is possible that additional unidentified oxidation derivatives of ascorbic acid may be formed since it has been reported that ascorbic acid is not quantitatively converted to oxalic and L-threonic acids by auto-oxidation (16).

The stability of ascorbic acid in urine and in solutions of various urinary salts has been studied by Lund and Lieck (17). These investigators found that the instability of ascorbic acid in phosphate was partially nullified by the presence of the chlorides of sodium, potassium, and ammonium. They also noted that when phosphate was present, uric acid and creatinine exerted a stabilizing action on ascorbic acid, whereas creatine and urea accelerated its auto-oxidation.

Compared with the *in vitro* breakdown of ascorbic acid, there is very little information on the nature of its breakdown in the animal body (18, 19).

In view of the existing reports on the subject, it appeared desirable to investigate the tuberculoinhibitory properties of ascorbic acid and its derivatives.

METHODS AND MATERIALS

Unless otherwise specified, the following methods and materials were employed throughout the investigations.

The mycobacteria used for bacteriostatic tests were *M. phlei*, *M. smegmatis*, the BCG and Ravenel strains of *M. tuberculosis* var. *bovis*, the H37Rv and H37Ra strains of *M. tuberculosis* var. *hominis*, and *M. tuberculosis avium*.

The organisms used for bacteriostatic tests were grown in Sauton medium at pH 7.0 for two days to two weeks. Suspensions were prepared by grinding some of the bacterial mass in a sterile mortar, washing in physiologic saline containing 0.04 per cent Tween 80[4] (Tween-saline) and, finally, resuspending in Tween-saline. The suspensions were diluted so as to contain 0.25 mg. wet weight of the bacteria per ml.

The tests for tuberculostatic activity were conducted with BCG.

The antibacterial tests were conducted in screw-cap tubes containing Dubos medium (20) plus 0.25 per cent bovine albumin fraction V or, in specified instances, in Proskauer and Beck medium at pH 7.0 (21) plus 10 per cent bovine serum. The ingredients in the final test mixtures consisted of 4.5 ml. of medium, 0.5 ml. of each of a series of various dilutions of the test material in physiologic saline containing M/60 phosphate buffer at pH 6.8 and 0.1 ml. of the suspension of the test organism. Control mixtures were of identical composition except that the test material was replaced by a similar volume of physiologic saline containing M/60 phosphate buffer at pH 6.8. Incubation of the preparations was carried out at 37°C. for one to two weeks with tubercle bacilli and for one to three days with the other organisms tested.

Crystalline L-ascorbic acid was used.[5] The solutions were prepared by adding the acid to M/60 phosphate buffer at pH 6.8. The pH was readjusted to 6.8 with 1N sodium hydroxide and sterilized by filtration through a Seitz filter. The auto-oxidation of the solutions of ascorbic acid was carried out by placing the material in shallow layers in lightly stoppered flasks at room temperature.

Reducing activity of the various materials was determined by the method of Van Eckelen and Heinemann (22).

RESULTS

Influence of Ingested Ascorbic Acid on the Tuberculostatic Property of Urine

In the first experiment, the urine of each of 2 healthy male subjects was collected at 10:00 p.m., and 1 gm. of ascorbic acid was immediately given by mouth. The total urine excreted during the succeeding eight hours was collected from each subject and titrated for bacteriostatic activity with the BCG strain of *M. tuberculosis* and with *M. phlei*. The titrations were performed in screw-cap tubes containing 2.0 ml. of Dubos broth to which 2.0 ml. of the various dilutions of urine in M/60 phosphate buffer at pH 6.8 were added. The experiment was repeated two weeks later on the same subjects with similar results.

The results of the first experiment showed that the increase in the reducing activity of urine following the ingestion of ascorbic acid was approximately tenfold for subject *A* and fifty fold for subject *B*. The increase in the tuberculostatic activity of urine for BCG following the ingestion of ascorbic acid was fivefold for subject *A*. In the case of subject *B*, the urine taken before treatment was not

[4] Tween 80 is the proprietary name for the polyoxyethylene derivative of Sorbitan monooleate, obtainable from the Atlas Powder Co., Wilmington, Delaware.

[5] The ascorbic acid was crystalline U.S.P. obtained from Merck & Co., Inc., Rahway, New Jersey.

tuberculostatic for BCG in a concentration of 50 per cent, whereas following treatment it was inhibitory in a concentration of 5 per cent. *Mycobacterium phlei* was not inhibited by concentrations of 50 per cent of urine of any of the urine samples. This is consistent with the subsequent observation that *M. phlei* was not inhibited to any appreciable extent by the addition of crystalline ascorbic acid or its derivatives to Proskauer and Beck medium.

It has been found (23) that normal subjects on a high ascorbic acid intake consistently show lower concentrations of the substance in night than in day urines, irrespective of the urine volumes. Hence, the physiologic "concentration" of urine which normally occurs during sleep would not be expected to account for the increased tuberculostatic activity of the urines of the experimental subjects following the ingestion of ascorbic acid. The rough correlation between the increase in reducing power of the urines and the increase in their tuberculostatic activity following the ingestion of ascorbic acid strongly suggests that the tuberculoinhibitory property of these urines was related in some way to their content of that substance.

The lack of an exact parallel between the reducing values of the urines and their tuberculostatic activity is not unexpected. Various amounts of reducing substances other than ascorbic acid may have been present in the urines even after deproteinization (22). Moreover, as shown later, the substance in urine which is capable of rendering culture media tuberculostatic appears to be a nonreducing derivative of ascorbic acid. Because of the last-named observation, there is no reason to expect that the urine should show an exact correlation between reducing power and tuberculostatic activity since the conversion of ascorbic acid to the inhibitory compound may take place to different degrees and at different rates in different urines. A correlation between the total original ascorbic acid and tuberculostatic activity would be a more reasonable expectation.

An experiment was next conducted to test the assumption that the increase in the tuberculoinhibitory property of urine following the ingestion of ascorbic acid was dependent on the acid and to ascertain by direct test whether ascorbic acid, when added to urine, will auto-oxidize *in vitro*.

> A sample of night urine of 350 ml. from a healthy subject was collected and filtered through a Seitz filter. Two aliquots of 45 ml. were then removed from the filtered sample. Five milliliters of a freshly prepared solution of ascorbic acid were added to one of the aliquots to give a final concentration of 500 γ per ml. The samples were incubated at 37°C., and tests of reducing and tuberculostatic activity were conducted at various intervals. The initial reducing activity was entirely absent after twenty-four hours. Tests of tuberculostatic activity for BCG showed the control urine to be negative in a concentration of 50 per cent at both the initial and twenty-four-hour intervals, whereas the urine with added ascorbic acid was tuberculostatic in a concentration of 10 per cent at the same intervals.

The results clearly showed that the addition of ascorbic acid to sterile urine renders it strongly tuberculoinhibitory and, moreover, that the tuberculostatic effect is unchanged even after the urine has lost its reducing ability. Although

TABLE 1

ANTIBACTERIAL SPECTRUM OF FRESH ASCORBIC ACID

ORGANISM	INCUBATION IN DAYS	ASCORBIC ACID IN γ PER ML.						
		0	10	20	40	60	80	100
M. tuberculosis BCG	7	4+	4+	4+	2+	—	—	—
M. phlei	5	4+	4+	4+	3+	3+	3+	2+
M. tuberculosis avium	5	4+	3+	1+	—	—	—	—
M. tuberculosis H37Rv	12	4+	3+	3+	2+	±	—	—
M. tuberculosis H37Ra	7	4+	4+	1+	—	—	—	—
M. smegmatis	5	4+	4+	4+	4+	4+	4+	2+
M. tuberculosis Ravenel	12	4+	4+	4+	3+	2+	2+	2+

Micrococcus aureus, *Proteus vulgaris*, *Corynebacterium diphtheriae*, and *Pseudomonas* species all showed growth equal to that of the control after twenty-four hours of incubation at 37°C.

Growth measured by visual comparison with control

Range: 4+ = maximum growth
1+ = scanty growth
− = no growth

these results alone do not establish that ascorbic acid itself is not tuberculoinhibitory, a subsequent study of the fate of ascorbic acid in the Proskauer and Beck test medium indicated that it undergoes extensive oxidation during periods shorter than the lag phase of growth for tubercle bacilli.

Tuberculostatic Activity of Ascorbic Acid

In order to extend the observations of others (6, 7) that the addition of ascorbic acid to culture medium renders it tuberculostatic, antibacterial tests were conducted.

Several species of bacteria were studied in Proskauer and Beck medium plus 10 per cent bovine serum to which various quantities of ascorbic acid were added, ranging from 10 to 200 γ per ml. The tubes were inoculated with 0.1 ml. of the suspension of each of the respective organisms containing 0.25 mg. wet weight of the bacteria per ml. The antibacterial spectrum obtained is presented in table 1.

The results show that the addition of ascorbic acid in concentrations of 20 to 40 γ per ml. rendered the medium tuberculostatic for *M. tuberculosis avium* and the BCG, H37Ra, and H37Rv strains of *M. tuberculosis*. Of the various genera tested, only organisms of the genus *Mycobacterium* were inhibited. It is notable, moreover, that the most marked activity of the tuberculoinhibitory substance was narrowly restricted to the pathogenic mycobacteria which included strains of both the avian and mammalian groups. A similar antibacterial spectrum has been reported by Bjornesjo (5) for the urinary principle he has isolated.

Fate of Ascorbic Acid in the Medium of Proskauer and Beck

Since the incubation interval necessary to obtain visible growth of tubercle bacilli under the cultural conditions used was at least five days, it was considered

important to study the fate of ascorbic acid in the medium in this long lag phase during which active derivatives might develop and exert their antibacterial action. Accordingly, an experiment was conducted in which the decrease in reducing activity was followed as an indication of the amount of ascorbic acid oxidized in Proskauer and Beck medium.

Ascorbic acid was added to Proskauer and Beck medium (without serum) at pH 7.0 to give a concentration of 100 γ per ml. Unfortunately, it was not feasible to employ the hydrazone test to measure the degree of oxidation of ascorbic acid in this medium since a copious nonspecific precipitate resulted from the action of the sulfuric acid present in this test reagent. However, the oxidation of ascorbic acid beyond the diketogulonic acid stage was followed quantitatively in a control solution of physiologic saline containing 100 γ per ml. of ascorbic acid in M/60 phosphate buffer pH 7.0 by employing the hydrazone procedure of Roe and Kuether (24). The mixtures were incubated at 37°C. and samples removed at intervals for determinations of reducing activity and capacity to form hydrazones.

The results on reducing activity, presented in figure 1, indicate that ascorbic acid undergoes rapid oxidation in Proskauer and Beck medium at pH 7.0. Similar results were obtained with ascorbic acid in M/60 phosphate buffer-saline at pH 7.0.

It is generally considered that diketogulonic acid is the principal material which reacts with 2,4-dinitrophenylhydrazine (12) since solutions of ascorbic acid, diketogulonic acid, and dehydroascorbic acid treated with 2,4-dinitrophenylhydrazine all yield hydrazones with identical melting points. This result can best be explained by assuming that ascorbic acid and dehydroascorbic acid are converted to diketogulonic acid during the reaction with the reagent.

The results of the hydrazone determinations in phosphate buffer are presented

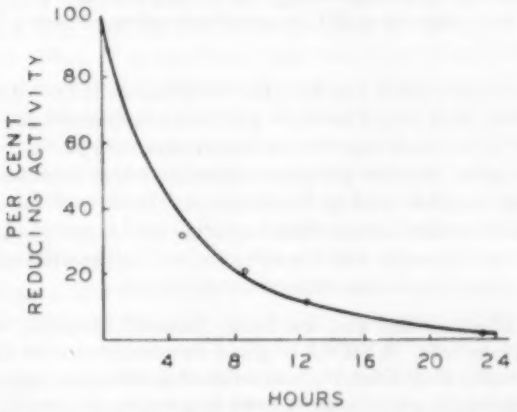

FIG. 1. The decline in reducing activity in a solution of 0.01 per cent ascorbic acid in Proskauer and Beck medium and in physiologic saline containing M/60 phosphate buffer at pH 7.0.

in figure 2. It is notable that, whereas after seventy-two hours the hydrazone-forming capacity of the solution had almost completely disappeared, its tuberculostatic activity for BCG was unimpaired. Full tuberculostatic activity was likewise noted after further oxidation when the material had lost all of its hydrazone-forming capacity.

These results clearly show that the tuberculoinhibitory derivative of ascorbic acid does not react with 2,4-dinitrophenylhydrazine. Moreover, it was noted in a subsequent experiment that, whereas a methyl alcohol precipitate obtained from an auto-oxidized solution of ascorbic acid was lacking in both reducing activity and hydrazone-forming capacity, it was fully tuberculoinhibitory. Still

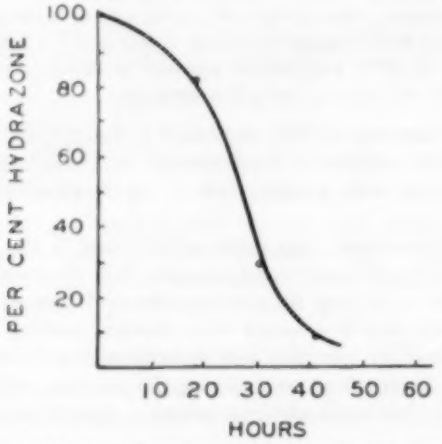

FIG. 2. The decline in hydrazone-forming capacity of a solution of 0.01 per cent ascorbic acid undergoing auto-oxidation at 37°C. in physiologic saline containing M/60 phosphate buffer at pH 7.0.

another relevant observation was that the tuberculostatic activity of Proskauer and Beck medium with added ascorbic acid was not diminished by preliminary oxidation at 37°C. for three days before inoculation with BCG.

The question arose whether the active principle which operates following the addition of fresh ascorbic acid to Proskauer and Beck medium is the same as that which operates when auto-oxidized ascorbic acid is added. Accordingly, an experiment was performed in which a comparative antibacterial spectrum analysis was made on fresh and auto-oxidized ascorbic acid.

The unoxidized solution used was freshly prepared by adding ascorbic acid to M/60 phosphate buffer at pH 6.8 to give a concentration of 50 mg. per ml. and filtering through a Seitz filter. The auto-oxidized material was prepared by allowing a similar solution to auto-oxidize at room temperature for several weeks until its hydrazone-forming capacity was lost. It was refiltered through a Seitz filter. The medium of Proskauer and Beck, pH 7.0, containing 10 per cent bovine serum, was used for the antibacterial tests.

TABLE 2

COMPARATIVE ANTIBACTERIAL SPECTRUMS OF FRESH AND AUTO-OXIDIZED ASCORBIC ACID

ORGANISM	INCUBATION IN DAYS	REAGENT	CONCENTRATION OF REAGENT IN γ PER ML.								
			0	10	20	40	60	80	100	200	400
M. smegmatis	4	AsA	4+	4+	4+	4+	4+	3+	3+	2+	1+
M. smegmatis	4	AA-AsA	4+	4+	4+	4+	4+	4+	4+	3+	3+
M. phlei	4	AsA	4+	4+	4+	4+	4+	4+	3+	3+	2+
M. phlei	4	AA-AsA	4+	4+	4+	4+	4+	4+	4+	4+	4+
M. tuberculosis avium	5	AsA	4+	3+	3+	3+	2+	2+	1+	—	—
M. tuberculosis avium	5	AA-AsA	4+	4+	4+	4+	3+	2+	1+	—	—
M. tuberculosis BCG	13	AsA	4+	4+	4+	4+	2+	1+	—	—	—
M. tuberculosis BCG	13	AA-AsA	4+	4+	4+	4+	3+	1+	1+	—	—
M. tuberculosis H37Ra	13	AsA	4+	4+	4+	2+	1+	±	—	—	—
M. tuberculosis H37Ra	13	AA-AsA	4+	4+	4+	3+	2+	2+	1+	—	—
M. tuberculosis H37Rv	13	AsA	4+	2+	2+	2+	1+	±	—	—	—
M. tuberculosis H37Rv	13	AA-AsA	4+	3+	2+	1+	±	±	—	—	—
M. tuberculosis Ravenel	13	AsA	4+	4+	3+	2+	2+	2+	±	—	—
M. tuberculosis Ravenel	13	AA-AsA	4+	4+	3+	2+	2+	2+	1+	—	—

Micrococcus aureus, *Proteus vulgaris*, *Corynebacterium diphtheriae*, and *Pseudomonas* species all showed growth equal to that of the control after twenty-four hours of incubation at 37°C.

AA-AsA = auto-oxidized ascorbic acid

Growth measured by visual comparison with control

Range: 4+ = maximum growth

1+ = scanty growth

— = no growth

The results presented in table 2 show that the antibacterial spectrums of fresh and auto-oxidized ascorbic acid in Proskauer and Beck medium are strikingly similar and indicate that the antibacterial principle concerned is the same for both materials. Thus, it seems likely that the active principle which operates following the addition of fresh ascorbic acid to Proskauer and Beck medium forms as the result of auto-oxidation. Moreover, the results also support the previous observation that the auto-oxidized material was fully as active as the fresh ascorbic acid. The somewhat lower values obtained with the auto-oxidized material in some instances in the present experiment may have resulted from some absorption of the active principle during a refiltration of the auto-oxidized solution which was carried out just before its use.

A comparison of the results in tables 1 and 2 shows that greater inhibition was obtained with fresh ascorbic acid in the first experiment shown in table 1 than in the second experiment shown in table 2. This may have resulted from unavoidable variations in the size of the inoculum of living organisms used, since in a subsequent experiment with BCG the use of approximately a tenfold inoculum greatly reduced the degree of inhibition; an effect which is commonly observed with many antimicrobials.

Tests on the Tuberculoinhibitory Activity of Ascorbic Acid Derivatives

As dehydroascorbic acid is known to mutarotate rapidly to diketogulonic acid at pH 7.0 (10, 11, 12), it appeared futile to attempt to isolate and determine the possible tuberculostatic properties of this substance.

Diketogulonic acid was isolated in the form of its calcium salt by the method of Penney and Zilva (12). In common with their observation it was noted that the salt, which is white on isolation, slowly turns a bright yellow color. Concentrations of the salt ranging from 10 to 200 γ per ml. were tested against BCG for tuberculostatic activity, using Proskauer and Beck medium plus 10 per cent bovine serum and M/150 borate buffer to stabilize the diketogulonic acid against oxidation. No tuberculostatic activity was noted in these preparations or in two control preparations without borate buffer. If, as has been reported, diketogulonic acid without stabilizer converts quantitatively to oxalic and L-threonic acids above pH 6.1 (12), the two latter derivatives would appear to be excluded as the tuberculoinhibitory substance present in medium to which ascorbic acid is added. Tests were next conducted with oxalic acid.

The maximum concentration of oxalic acid theoretically attainable from the minimum tuberculostatic concentration of ascorbic acid (20 γ per ml.) is approximately 10 γ per ml. An experiment was conducted in which concentrations of oxalic acid ranging from 10 to 200 γ per ml. were added to Proskauer and Beck medium plus 10 per cent bovine serum and inoculated with BCG in the usual manner. Tuberculostatic activity was entirely lacking for all of the concentrations employed. Thus, it appears that oxalic acid is not the tuberculostatic agent operating in media to which ascorbic acid is added.

Tests were not conducted with L-threonic acid.

The possible tuberculostatic activity of furfurol was next tested in Proskauer and Beck medium plus 10 per cent bovine serum, using concentrations which ranged from 10 to 200 γ per ml. The results of this experiment likewise failed to show any tuberculostasis.

Hydrogen peroxide has been reported to be formed in the course of oxidation of ascorbic acid (25, 26) and is regarded by Lwoff and Morel (26) to be the antibacterial agent which forms in ascorbic acid solutions.

In order to test this possibility, several experiments were conducted in which 10 to 100 γ per ml. of ascorbic acid were incorporated in Proskauer and Beck medium plus 10 per cent bovine serum and catalase supplied by the addition of 0.05 to 0.1 per cent of hemolyzed rabbit erythrocytes. The catalase activity of the medium was confirmed at the beginning and end of the incubation period by the addition of a few drops of 3 per cent hydrogen peroxide to the control tubes. The results showed that the inhibition of BCG was as marked in the catalase-containing series as in the control series. Thus, it appears that hydrogen peroxide is not the active tuberculoinhibitory derivative of ascorbic acid which is being sought.

The foregoing results give no indication that either ascorbic acid or any of the known derivatives occurring in the classical breakdown of ascorbic acid are tuber-

culostatic, and completely fail to account for the tuberculostatic property imparted to culture media by the addition of ascorbic acid.

Another possibility considered was that the antibacterial principle might be a contaminating substance in the preparation of ascorbic acid used. Accordingly, a recrystallization of the ascorbic acid was carried out by dissolving 10 gm. in 20 ml. of distilled water with the aid of heat. The warm solution was filtered and cooled in an ice bath. Approximately 1 gm. of ascorbic acid crystallized out; this was collected, washed, and employed for assay purposes against BCG. Even though 90 per cent of the ascorbic acid was discarded in the process of recrystallization, the tuberculostatic activity was unchanged.

Sorbitol, the material commonly used for the commercial preparation of ascorbic acid, showed no antibacterial activity against BCG when added to Proskauer and Beck medium in concentrations of 10 to 400 γ per ml.

Chemical Properties of the Active Principle

An experiment was conducted in which a 5 per cent solution of ascorbic acid in M/60 phosphate buffer at pH 7.2 was allowed to auto-oxidize for a period of eight weeks at room temperature. Although the resulting dark amber solution was found to be completely devoid of its original reducing activity, full tuberculostatic activity remained. As a test of the heat stability of the principle, samples of the solution were adjusted to pH values of 1.4, 7.0, and 12.4 with 1 N hydrochloric acid and 1 N sodium hydroxide. The solutions were autoclaved for thirty minutes at 120°C, adjusted to pH 7.0, and appropriate dilutions were made in M/60 phosphate buffer at pH 7.0 for tests of tuberculostatic activity. The results showed the heated samples to be fully as tuberculostatic as the unheated controls and establish that the active principle has remarkable heat stability.

An aliquot of the solution was treated with 10 volumes of absolute methyl alcohol, and the resulting precipitate was washed several times in methyl alcohol and several times in ether. The precipitate was dried and dissolved in distilled water. Although this solution was completely lacking in reducing activity and the capacity to form a hydrazone, it was found to contain 90 per cent or more of the tuberculostatic activity present in the original aliquot of the auto-oxidized solution. When the clear supernatant solution remaining after the methyl alcohol precipitation was evaporated in a stream of nitrogen gas and the resulting residue reconstituted with buffer and titrated against BCG, less than 1 per cent of the tuberculostatic activity of the original aliquot of the auto-oxidized solution was found.

The methyl alcohol precipitate was found to be insoluble in acetone, ether, petroleum ether, benzene, carbon tetrachloride, and chloroform. It was sparingly soluble in 95 per cent ethanol and completely soluble in 80 per cent ethanol.

Attempts to characterize the substance by ultraviolet light spectrum analysis did not reveal any absorption peaks which could be identified.

The properties of the substance do not identify it with ascorbic acid or any of the known derivatives of ascorbic acid. The similarity of its solubility and anti-

bacterial properties to those of the urinary substance of Bjornesjo (3–5) suggests that the two substances may be identical.

An experiment was next conducted on a solution of ascorbic acid oxidized to dehydroascorbic acid with an equivalent amount of iodine. Prior to precipitation with the various reagents below, the solution was adjusted to pH 7.2 and allowed to undergo further oxidation at room temperature for seven days until it turned a dark amber color. As the respective precipitates obtained by the addition of absolute ethanol, 95 per cent ethanol, absolute methanol, and acetone were all found to lack tuberculostatic activity, it seems probable that the active derivative which results from auto-oxidation is formed directly from ascorbic acid by some pathway other than that of dehydroascorbic acid and diketogulonic acid.

SUMMARY

Studies on ascorbic acid and its various derivatives indicate that the tuberculostasis which is effected by the addition of ascorbic acid and auto-oxidized ascorbic acid to culture medium is due to an auto-oxidation product of ascorbic acid of unknown chemical composition.

A methyl alcohol precipitate has been prepared from auto-oxidized solutions of ascorbic acid which is tuberculostatic. The active principle in the precipitate is a heat-stable, nonreducing, nonhydrazone-forming substance which is soluble in water and in 80 per cent ethanol. It is insoluble in acetone, ether, petroleum ether, benzene, carbon tetrachloride, and chloroform. As the substance is formed when ascorbic acid is allowed to undergo auto-oxidation but not when oxidized with iodine, it is suggested that the substance may be formed by some pathway other than by way of dehydroascorbic acid and diketogulonic acid.

It was observed that, when ascorbic acid was added to the sterile filtered urines of healthy human subjects, it underwent rapid oxidation as indicated by the loss of reducing activity. Both these urines and the urines of human subjects taken after the ingestion of large quantities of ascorbic acid possessed greatly enhanced tuberculostatic activity.

The antibacterial and chemical properties of the active principle in auto-oxidized solutions of ascorbic acid are similar to those of the tuberculostatic principle of urine described by Bjornesjo.

SUMARIO

Estudios de las Propiedades Tuberculoinhibidoras de los Derivados del Acido Ascórbico y de su Posible Papel en la Inhibición de los Bacilos Tuberculosos por la Orina

Estudios del ácido ascórbico y de sus varios derivados indican que la tuberculostasis que tiene lugar al agregar a los medios ácido ascórbico y ácido ascórbico autooxidado se debe a un producto de la autooxidación del ácido ascórbico, cuya composición química es desconocida.

De las soluciones autooxidadas del ácido ascórbico se ha preparado un precipitado en alcohol metílico, que es tuberculostático. El principio activo del precipitado es una substancia no hidrazonógena, no reductora, termoestable, que es soluble en agua y en etanol al 80 por ciento, siendo insoluble en acetona, éter, éter de petróleo, benceno, tetracloruro de carbono y cloroformo. Como la substancia se forma cuando se deja que el ácido ascórbico experimente autooxidación, pero no cuando se oxida con yodo, parece que la substancia se forma por alguna vía distinta de la del ácido dehidroascórbico y del ácido diquetogulónico.

Observóse que, cuando se añadía ácido ascórbico a las orinas filtradas estériles de sujetos humanos sanos, experimentaba rápidamente oxidación, según indicaba la pérdida de actividad reductora. Tanto dichas orinas como las obtenidas de sujetos humanos consecutivamente a la ingestión de grandes cantidades de ácido ascórbico mostraban una actividad tuberculostática muy realzada.

Las propiedades antibacterianas y químicas del principio activo contenido en las soluciones autooxidadas del ácido ascórbico son similares a las del principio tuberculostático de la orina descrito por Bjornesjo.

RESUME

Etude des propriétés tuberculostatiques de dérivés de l'acide ascorbique et leur rôle possible dans l'inhibition du bacille tuberculeux par l'urine

Les études sur l'acide ascorbique et ses divers dérivés indiquent que l'action tuberculostatique, effectuée par l'addition au milieu d'acide ascorbique et d'acide ascorbique auto-oxydé, est due à un produit d'auto-oxydation de l'acide ascorbique dont l'origine chimique est inconnue.

Un précipité méthyl-alcool préparé à partir d'une solution d'acide ascorbique auto-oxydé a une action tuberculostatique. Le principe actif du précipité est une substance stable, non réductrice et ne formant pas d'hydrazone; cette substance est soluble dans l'eau et dans l'éthanol à 80%, insoluble dans l'acétone, l'éther, l'éther de pétrole, le benzène, le tétrachlorure de carbone et le chloroforme. Comme cette substance est formée lorsqu'on laisse l'auto-oxydation de l'acide ascorbique se produire, mais fait défaut lorsque l'oxydation est produite par l'iode, les auteurs supposent que cette substance pourrait être formée par une autre voie que celle de l'acide déhydroascorbique et de l'acide dicétogulonique.

Il a été observé que si on ajoute l'acide ascorbique à l'urine stérile filtrée d'un sujet bien portant il s'oxyde rapidement, comme le montre la perte de son activité réductrice. Ces urines, comme celles provenant d'individus bien portants prélevées après ingestion d'une grande quantité d'acide ascorbique, possèdent un pouvoir tuberculostatique considérablement augmenté.

Les propriétés antibactériennes et chimiques du principe actif renfermé dans les solutions d'acide ascorbique auto-oxydé sont semblables à celles du principe tuberculostatique décrit par Bjornesjo.

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EXPERIMENTAL EMBOLIC PULMONARY TUBERCULOSIS IN MICE[1]

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Introduction

The present study deals with the experimental tuberculosis in mice which is used for drug screening and evaluation tests in these laboratories. In developing the screening method, a particular type of bacterial suspension was selected because its intravenous injection yielded highly reproducible survival times in untreated mice (1). Although these screening tests were based on survival time only, various considerations made it advisable to study the morphology of mouse tuberculosis as produced in these laboratories.

The morphologic studies of the mice revealed the surprising fact that pulmonary emboli, consisting of clumps of tubercle bacilli, were produced by the intravenous injections. Morphologic examination of the suspensions used for injection showed that they contained bacterial clumps larger than the diameter of pulmonary capillaries and arterioles. The size of bacterial clumps and their number in the standard dose were not large enough, however, to cause any visible disturbance of the circulation in the lung. Because of these favorable circumstances, the "embolic pulmonary tuberculosis" produced offered a new opportunity for studying the establishment and progress of an experimental tuberculous infection of the lungs. Such opportunity was welcome, since the early phases of pulmonary tuberculosis had never been demonstrated by those investigators who injected well-dispersed suspensions intravenously or employed the respiratory route of infection.

The fate of the emboli which serve as pulmonary inocula and the subsequent development of the tuberculous lesions will be described in the present paper. The relation between survival times and the morphologic processes in the lungs will be discussed. The emphasis will be on the pulmonary processes, since the other organs showed a relatively slight tuberculous involvement.

Material and Methods

Bacterial cultures and suspensions used for injections[2]: According to the routine method of the Bacteriology Laboratory, two strains of *M. tuberculosis* were cultivated on the surface of Kirchner's medium, namely, D4M3 (bovine type) and H37Rv (human type).[3] The growth on Kirchner's medium, the preparation of suspensions of D4, and the definition of doses by turbidity units were described in 1949 by Baker, Schlosser, and White (1). Therefore, only certain modifications which have developed since will be mentioned here. Until

[1] From the Chemotherapy Division, Stamford Research Laboratories, American Cyanamid Company, Stamford, Connecticut.

[2] Details of the bacteriologic techniques can be obtained on request from Dr. H. J. White of the Bacteriology Laboratory of this Division.

[3] Subsequently the abbreviations D4 and H37 will be used to designate the two strains.

June 12, 1951, pellicles from three-week-old cultures had been used throughout (first 4 experiments of table 1), while, subsequently, pellicles from two-week-old cultures were used (last 4 experiments of table 1). The pellicles were blended in a Waring Blendor at all times; additional grinding in a Ten Broeck homogenizer was abandoned after July 5, 1951. Finally, the strain H37, which previously had been found unsatisfactory for routine tests at Stamford, proved to give reproducible results after some modifications in preparing the suspensions.

The material obtained from the pellicles by blending was diluted with Kirchner's medium to the turbidity desired. The standard suspension, which kills 50 per cent of the mice in fourteen days, has a turbidity of 80 for the strain D4 and a turbidity of 160 for the strain H37. The suspension of D4 was aspired immediately after stirring and diluted to turbidity 80. The suspension of H37 was allowed to settle for two minutes after stirring; then, only the supernatant part was aspired and diluted to turbidity 160.

The volume injected into a tail vein of each mouse was always 0.2 ml. Therefore, the standard dose was defined as 0.2 ml. of the standard suspension; it was equivalent to approximately 0.1 mg., dry weight, or 0.5 mg., wet weight, of bacilli. Experiments with five different doses required highly concentrated suspensions from which the lower concentrations were obtained by dilution. When 1x represents the standard dose of strain D4 with the turbidity 80, the dose 32x had a turbidity of approximately 2,600, the dose 16x of approximately 1,280, and the doses 4x, x/4, and x/16 had turbidities of 320, 20, and 5, respectively. No difficulty was encountered in injecting even the most concentrated suspensions into a tail vein.

It is not easy to study two-week-old pellicles in total mounts because of their density and brittleness; therefore, samples of them were embedded in paraffin, sectioned, and stained. Delicate pellicles were produced by repeated transfers at short intervals, e.g., every three or four days; pieces of such young cultures could be affixed to a slide and stained for acid-fast and nonacid-fast bacilli (figure 3). There was no difficulty in making total mounts from the suspensions which were obtained by blending two- or three-week-old pellicles. For morphologic study, drops of a suspension were placed on a slide and spread by gravity or gentle stirring; after drying, the preparations were stained with carbolfuchsin, differentiated with acid-alcohol, and counterstained with methylene blue (figure 4).

Mice: Vanderwerken mice were used for the first 7 experiments listed in table 1, while Carworth Farm mice (CF_1) were used in the last experiment. The mice were six or seven weeks of age, weighing 18 to 22 gm. at the beginning of the experiments. After arrival at the laboratory all mice were quarantined for a week and used for experiments only when they appeared to be in good health. Routinely, 10 mice were kept in one cage.

Injections, sacrifices, and morphologic procedures: The suspensions of tubercle bacilli were injected into the tail veins of mice in the routine way. For morphologic studies, mice were sacrificed with illuminating gas five minutes to fourteen days after injection. Mice which were found dead were excluded from morphologic study. Mice which died in the presence of the pathologist, either from rapidly killing high doses or because they had reached the fatal stage of the standard infection, were suitable for use. The autopsies included the thoracic and abdominal cavities in all series and the cranial cavity in one series.

The lungs of all mice were preserved for morphologic study. For optimal fixation the pulmonary lobes were placed separately into the fixative, but for special purposes the chest organs were fixed as a whole. When the lobes had been separated, every left lobe, being the largest, was examined histologically on longitudinal sections, while the number of right lobes examined varied.

In addition, the following organs were studied in a limited number of mice: spleen, liver, kidneys, adrenals, heart, brain, and femoral marrow; the femur was cracked gently in several places to allow fixation of the marrow. After paraffin embedding, sections of 6μ or 3μ thickness were made. Serial sections were studied whenever necessary.

From every block of tissue several sections were stained with carbolfuchsin, differentiated with acid-alcohol, and counterstained with hematoxylin. In the illustration text this

procedure will be referred to as "double stain." In many cases the following combination was used for lung sections: carbolfuchsin, incomplete differentiation with acid-alcohol, Weigert's blue elastica stain (resorcin fuchsin), differentiation with 95 per cent alcohol, hematoxylin. This combination will be briefly termed "triple stain."

The extensive photomicrographic work was done in cooperation with E. J. Thomas of the Microscopy Laboratory, Analytical and Testing Division of these laboratories. The present paper contains a limited selection of photomicrographs; on request, additional pictures from the file can be obtained for documentation of special points.

SURVIVAL TIMES

In mice which lived for more than twenty-four hours after injection, the survival time was recorded by the routine "census." However, survival times could not be evaluated statistically in the groups of mice which served for morphologic study, since some of the mice were sacrificed and others died of the infection. Therefore, each morphologic study was accompanied by one or two groups of mice (10 in each group) which were injected with the same suspension and served as "survival controls" without any interference by sacrifices.

In the 1949 paper of Baker, Schlosser, and White (1), charts had been shown (*a*) of the median survival times of 97 consecutive groups of 10 mice each which had been infected with the standard dose of D4, and (*b*) of the distribution of the

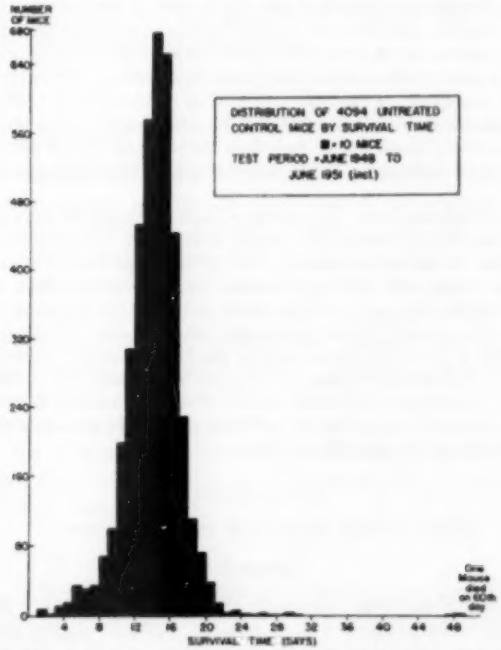

NUMBER OF MICE
DISTRIBUTION OF 4094 UNTREATED CONTROL MICE BY SURVIVAL TIME
■ = 10 MICE
TEST PERIOD = JUNE 1948 TO JUNE 1951 (incl.)
One Mouse died on 60th day
SURVIVAL TIME (DAYS)

FIG. 1. Distribution by survival time of control mice infected with standard dose of tubercle bacilli, strain D4.

pool of all of these mice by survival time; these charts covered experiments from June, 1948, to June, 1949. The graph shown in figure 1 of the present paper covers two additional years of injections, i.e., the total period from June, 1948, to June, 1951, and a total of 4,094 mice. The mice of the first 4 experiments of the present paper, listed in table 1, are included in those of the graph (figure 1).

In the paper by Baker, Schlosser, and White (1), the survival times of groups of 10 mice were calculated by plotting the cumulative percentage mortality against time in days after infection, on logarithmic probability paper, and by determining the death of 50 per cent of the mice by means of the rapid graphic method of Litchfield (2). In the present study, the same procedures have been used for calculating the times (in days) when 16 per cent, 50 per cent, and 84 per cent of the mice were dead. The days obtained in this way are referred to as "calculated values" of survival times in tables 1 and 3. In addition, the actual ranges of survival time are given in these tables.

Experimental

A total of 400 mice were injected with suspensions of tubercle bacilli in the experiments presently reported. For morphologic studies, 80 mice were injected with the standard dose of strain D4 and 20 mice with the standard dose of strain H37. Since mice found dead could not be used, 55 mice injected with D4 and 16 mice injected with H37 remained available for investigation; these 71 mice are listed individually in table 2. For survival tests in parallel to the morphologic studies, 110 mice were injected with the standard dose of strain D4 and 20 mice with the standard dose of strain H37; these 130 mice are listed by groups in table 1. The D4 experiments covered the period from February 2, 1950, to February 11, 1952, while the H37 experiment was made on September 8, 1952. A comparison of tables 1 and 2 shows that there were 8 separate survival tests, but only 7 morphologic studies with corresponding series numbers; this is explained by the fact that the survival test of September 12, 1951, (Series 13 L Contr.) was preliminary to the morphologic study and survival test of October 18, 1951.

In addition to the standard dose (1x), different doses of strain D4 were injected for survival tests in 3 of the 7 experiments mentioned above. For this purpose, 170 additional mice were used (listed by groups in table 3). Two of the experiments were made with the doses 16x, 4x, 1x, x/4, and x/16, while in the third experiment the doses 32x and 1x were given. Seven mice which were part of these survival tests were also used for morphologic study; they died in the presence of the pathologist after injection of 16x or 32x.

Histologic studies of the lungs were made in the 71 mice which received the standard dose (table 2) and in the selected 7 mice injected with the doses 16x or 32x. Organs other than the lungs were examined in a limited number of mice at various intervals after injection of the standard dose of strain D4. In addition, the hearts were examined in the 7 selected mice which received the doses 16x or 32x.

Observations

Experiments with the standard dose

Survival

The results of the 8 survival tests with the standard dose are seen in table 1. The 7 experiments with strain D4 were spaced over two years; they showed the uniformity of survival times which, for many years, has been a characteristic of these tests in the Bacteriology Laboratory. The eighth survival test (Series

TABLE 1

SURVIVAL TIME OF MICE INFECTED WITH STANDARD DOSE OF STRAIN D4 OR H37 (IN PARALLEL WITH MICE INFECTED AND SACRIFICED FOR MORPHOLOGIC STUDY)

DATE OF INFECTION	STRAIN OF BACILLI	SERIES NUMBER	NUMBER OF MICE*	SURVIVAL TIME IN DAYS			
				Actual Range	Calculated Values†		
					16 Per Cent Dead	50 Per Cent Dead	84 Per Cent Dead
February 2, 1950	D4	3 L Contr.	20	4–18	11	13	15
December 21, 1950	D4	5 L Contr.	20	5–15	8	10	12
January 11, 1951	D4	8 L Contr.	10	9–18	10	12	15
February 22, 1951	D4	10 L Contr.	20	4–15	9	11	14
September 12, 1951	D4	13 L Contr.‡	20	8–18	10	12	15
October 18, 1951	D4	11 L Contr.‡	10	9–17	10	13	17
February 11, 1952	D4	16 L Contr.‡	10	9–17	11	13	15
September 8, 1952	H37	20 L Contr.	20	7–18	10	13	16

* At termination of experiments, all mice had died.
† Calculated according to Litchfield's method (2).
‡ Identical with those in table 3 which received the dose 1x.

20 L Contr.), made with the standard dose of strain H37, produced survival times similar to those of the D4 experiments.

Morphology

Morphology of Suspensions

Stained preparations of drops of suspensions contained closely packed clumps of bacilli of various sizes, aggregates of few bacilli, and single bacilli. Figure 4 shows an assortment of clumps and also illustrates the fact that a clump may break up into smaller fragments and that small aggregates may be pushed together to form a bigger unit. The 2 largest clumps in the picture measure approximately 25 x 25 μ and 45 x 20 μ, respectively, but clumps of 50 x 30 μ in diameter are not rare in the suspensions of either strain. Stained sections of two-week-old pellicles (before blending) showed a parallel arrangement of bacilli similar to that in total mounts of young pellicles (figure 3).

Although no sections of clumps from blended suspensions were made, there is no doubt that the clumps in the suspensions are fragments of the cords which compose the pellicles. Occasionally, snakelike fragments of a thin cord may be found in the blended suspensions.

Morphology of Lungs

Sequence of events: The phenomena observed in the lungs are presented in diagrams *a* through *g* of figure 2, with descriptions in the figure text. Each diagrammatic picture is documented by one or more photomicrographs (figures 5 through 20). Only a few points need explanation.

The small pulmonary arteries (arterioles) consist of muscularis, elastica, and

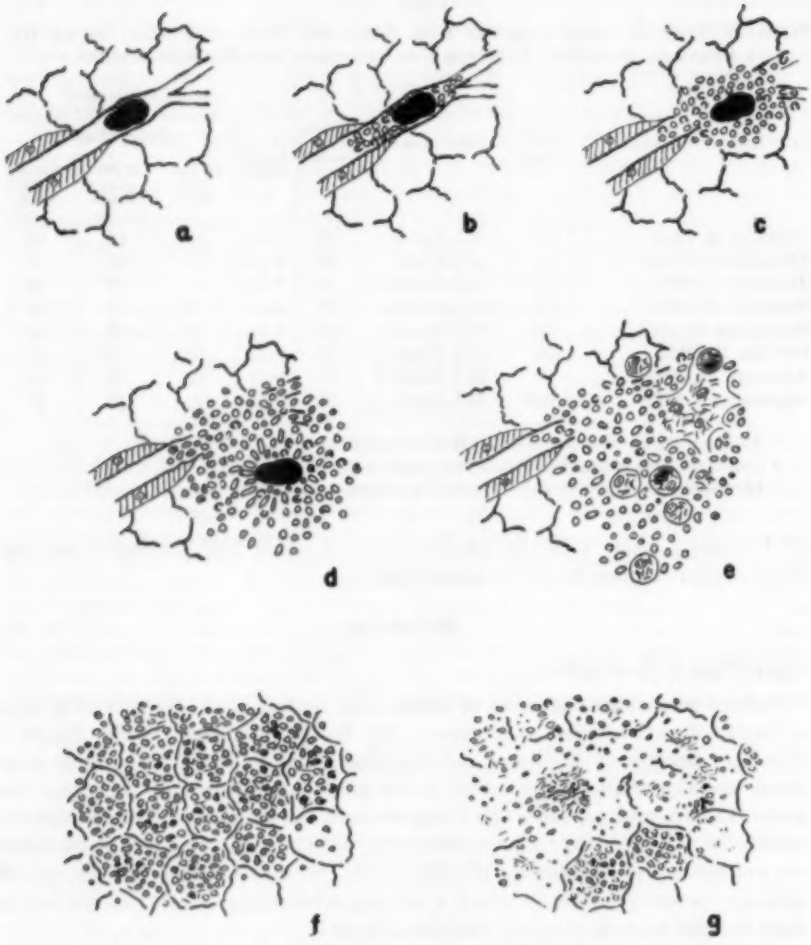


FIG. 2. Diagrams of stages of embolic pulmonary tuberculosis. (*a*) Small pulmonary artery with typical sudden change from thick wall (shaded) to thin wall; bacterial clump (black) arrested in thin-walled part. (*b*) Intravascular cellular reaction around bacterial embolus. Fifteen minutes after injection or later. (*c*) Bacterial clump plus reacting cells breaking through elastica of vessel. One hour after injection or later. (*d*) Reactive nodule, with bacterial clump in its center, located in the alveolar tissue. Usually, twenty-four hours after injection. (*e*) Bacterial clump dispersed by mononuclear phagocytes (which are drawn unproportionally large to show bacilli). Start at twenty-four hours or later, fully developed usually after four days. (*f*) Tuberculous pneumonia; alveolar pattern still preserved. Fully developed after ten days or later. (*g*) Massive necrosis; alveolar pattern destroyed (except on lower margin of picture); debris of cells and elastica, teeming with bacilli. Fully developed after twelve days or later.—*Note:* topographically, stages (*f*) and (*g*) are no longer related to embolized vessels, but may develop anywhere in the lungs.

endothelium. In mice as well as in other species, these vessels are peculiar in that the thickness and composition of their walls may change in an abrupt way. In such places the muscular layer does not decrease gradually but disappears almost suddenly so that the new segment has a much thinner wall consisting of elastica and endothelium only.[4] Decrease in wall thickness is not necessarily connected with a decrease in bore (figure 8). If a bacterial embolus is arrested in the thick muscular part of a vessel, the cellular reaction will remain intravascular for a long time. If, on the other hand, the embolus lodges in a thin-walled part, the intravascular reaction may lead to a perforation of the elastica as early as one hour after injection of the suspension. Very small emboli in narrow capillaries seem not to break through that early, probably because of the small number of cells they attract.

The diagrams *a* through *e* of figure 2 illustrate the fate of a large embolus which had been arrested in a thin-walled segment of an arteriole. Evidently, the ruptured wall of a vessel is well plugged by the embolus and the surrounding cells since remnants of hemorrhage are rare and small (figure 10).

Usually, twenty-four hours after injection the reactive nodules are fairly discrete, irrespective of their production by large clumps or by tiny aggregates of bacilli (figures 17 and 19). Subsequently, the nodules not only increase in size but fuse together (not shown in the diagrams of figure 2); their distinctness decreases more and more when the bacillary clumps are dispersed by monuclear phagocytes (figures 2 *e* and 11).

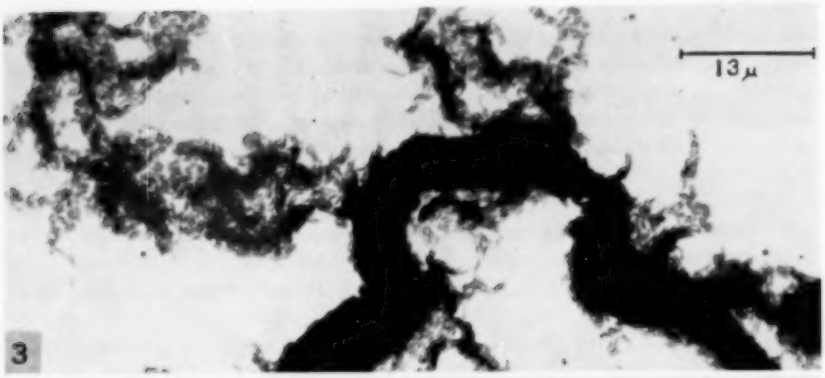
Approximately four days after injection, phagocytes laden with bacilli may be found all over the lung, and the subsequent stages (diagrams *f* and *g* of figure 2) are topographically no longer related to embolized vessels and bacillary clumps. Tuberculous pneumonia, with or without massive necrosis but always teeming with bacilli, is the final and fatal stage, in which 50 per cent or more of the pulmonary tissue is consolidated. The macroscopic appearance of the lungs in the final stages is seen in figure 14. The volume of largely consolidated tuberculous lungs is similar to that of expanded normal lungs (figures 13 and 14).[5]

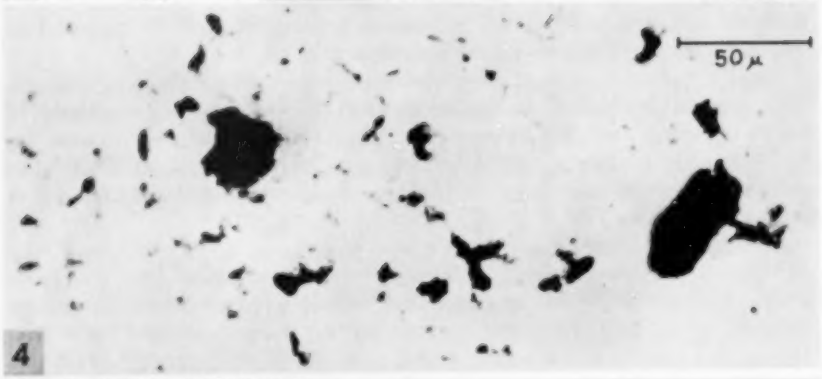
The diagrams of figure 2 are arranged as a sequence of several phases. The actual observations from which the sequence of events has been reconstructed are listed in table 2, which contains the findings in the lungs of each of 71 mice, with the intervals after injection indicated.

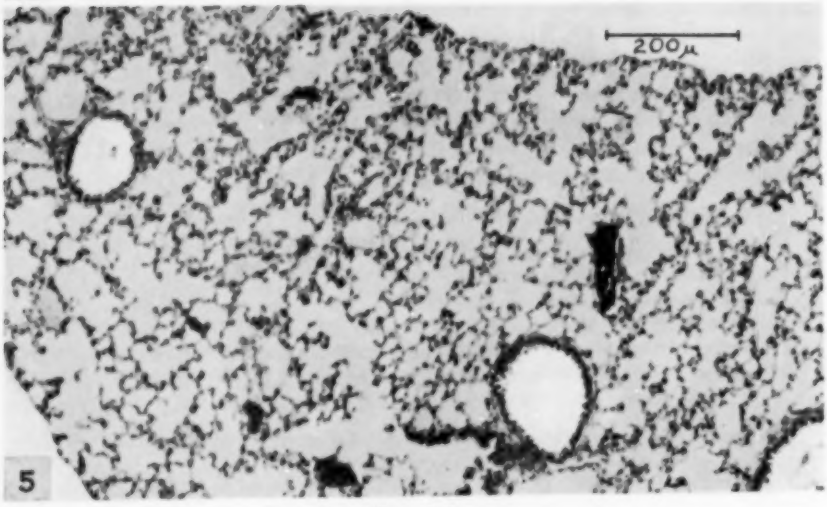
The entries in the columns of the table are: *present* (+), *doubtful* (±), *absent* (−), or *not examined* (blank). The heading "Large Clumps of Tubercle Bacilli" covers both emboli in vessels and former emboli in reactive nodules; the presence of any clumps equaling or exceeding 20 x 10 μ in diameter is indicated by +, while ± is used if only smaller clumps were observed. In the column "Rupture of Vascular Elastica," the absence of an entry may mean two different things: either no ruptures were seen in elastica-stained sections, or no elastica-stained sections were available. Under the heading "Extravascular Nodules," discrete and fused nodules have been combined.

[4] We are greatly indebted to Dr. Arnold R. Rich of Johns Hopkins Medical School for having attracted our attention to this anatomic phenomenon.

[5] Unfortunately, most publications use pictures of collapsed normal lungs for a comparison, thus creating the impression that tuberculous lungs are "enlarged."







FIGS. 3–5

It is readily seen that the plus signs of table 2 move from the left upper part to the right lower part of the table. This is the basis for the chronologic interpretation of the findings and of the diagrammatic presentation in figure 2.

Variation of embolized material from standard suspension: The variation of emboli in the lungs can only be studied before their dispersion by macrophages has started. Since it is impossible to put the variation of embolized material on a quantitative basis, this part of the study was limited to the determination of the maxima and minima of embolic masses observed in the mice of table 2. The most interesting information was obtained through the experiment with strain H37 (Series 20).

Two mice of this series, which were sacrificed twenty-four hours after injection, happened to represent maxima and minima of embolic material. The maximum is illustrated in figure 17, while figure 19 illustrates the minimum. These 2 mice had received identical amounts, which were part of the same charge of a syringe. Similar maxima and minima occurred among the mice injected with strain D4. Here, the maximum of emboli, both with respect to size and number, was observed in Mouse 3 of Series 3 L, sacrificed four days after injection; the histologic picture was comparable to figure 17. The minimum of embolic material in the D4 experiments was seen in Mouse 9 of Series 16 L, sacrificed two days after injection; the microscopic picture resembled figure 19, including the size of the small, solitary clump.

Morphology of Organs Other Than the Lung

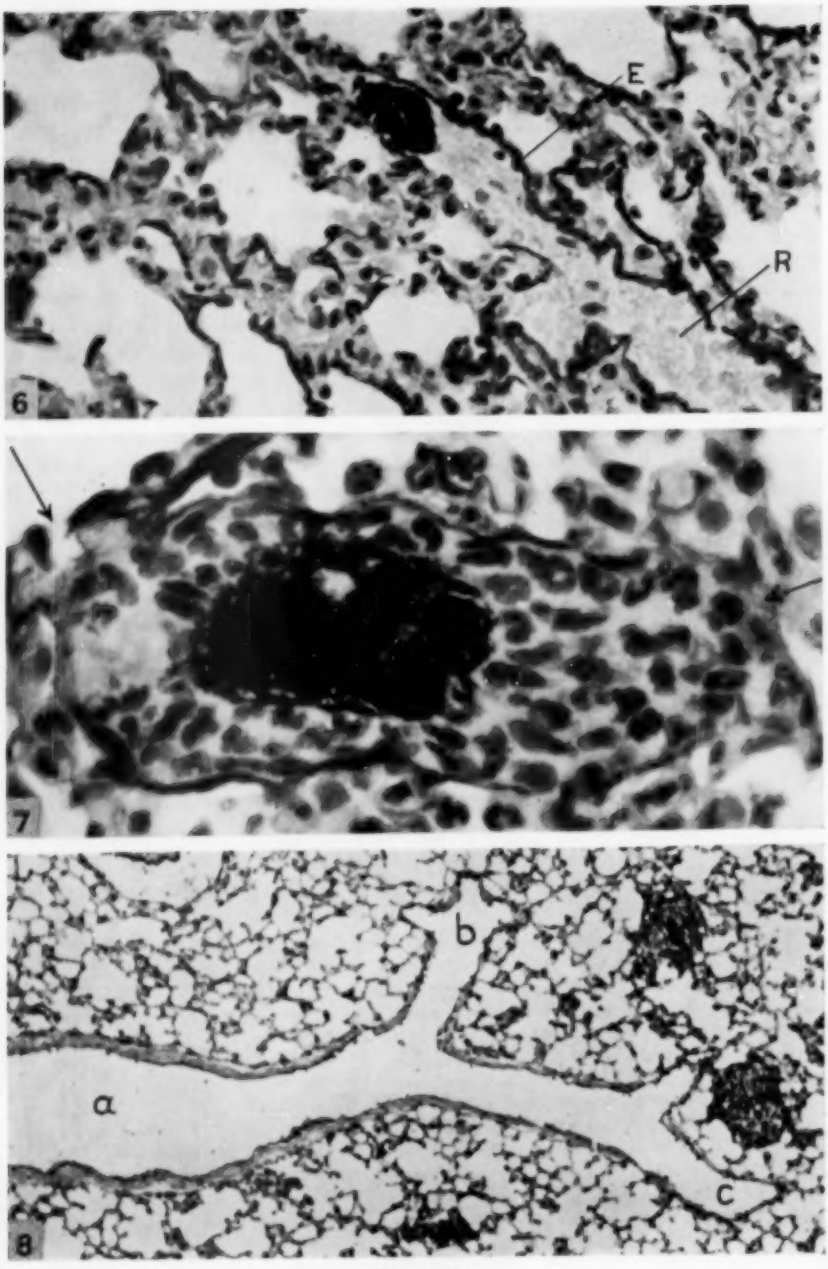
Other than the lungs, the following organs were examined at various intervals after injection of strain D4, standard dose: spleen, liver, and kidneys from three hours to thirteen days in 20 mice; the femoral marrow from six to twelve days in 4 mice; the adrenals and brain on the twelfth and fourteenth days in 5 mice; and the hearts from five minutes to four days in 5 mice and on the twelfth and fourteenth days in 5 mice.

Tubercle bacilli were, at one time or another, found in representatives of each of the 7 organs. Between three and twelve hours after injection, 6 of 6 spleens and 5 of 6 livers showed tubercle bacilli, which were either scattered or formed small aggregates. Clumps of the size seen in the lungs (20 x 10μ or more) were

FIG. 3. Preparation from a three-day-old pellicle of strain D4, Kirchner's medium, last of 7 "short transfers" (three- or four-day intervals for twenty-three days). Acid-fast densely packed, thick cords appear black in the picture, nonacid-fast delicate fringes or cords appear gray, with admixture of single acid-fast bacilli appearing black; dark gray appearing bacilli are semi-acid-fast ("purple"). Sample dried on slide without smearing or stirring, coated with celloidin, stained with carbolfuchsin, differentiated with acid alcohol, counterstained with Löffler's methylene blue, mounted in clarite. (×1,500)

FIG. 4. Preparation from a suspension of a three-week-old pellicle of strain D4; this suspension was used for injecting Series 10 L. Great variability in size of clumps. Stain as in figure 3. (×400). *Note*: Preparations from suspensions of strain H37 appear similar.

FIG. 5. Series 16 L, Mouse 5; strain D4, five minutes after injection of standard dose. Lung showing embolic clumps (black) of different sizes. 6μ section, double stain. (×100) *Note*: From the 5 lobes examined, this area was selected for relatively high amount of emboli.



FIGS. 6–8

never observed. Particularly noteworthy was the presence of a few bacilli in the myocardium (Series 16 L, Mouse 5) as early as five minutes after injection.

The lesions found in any of the 7 organs were very small and scarce, compared with those in the lungs. In the advanced stages of the disease, small cellular foci containing numerous bacilli and showing signs of necrosis were occasionally found in the myocardium, the liver, the adrenals, and the brain. Although tubercle bacilli were seen in every femoral marrow examined (six to twelve days after injection), appreciable cellular reactions or necrosis was lacking. During the last stages of the disease, the spleens showed the well-known enlargement with loss of the normal microscopic pattern, but without typical tuberculous lesions (cf. Stewart (3)).

EXPERIMENTS WITH DIFFERENT DOSES

Survival

The results of the 3 survival tests with different doses are seen in table 3. With the use of dose *16x* or *32x*, many mice died within a few seconds or minutes after injection, but the ratio of mice dying that fast varied and some mice survived for several days. Even with the dose *32x*, 2 mice survived for three days (Series 16 L, Mice 15 and 16). It is evident that the survival times with these high doses could not be handled statistically. The data of table 3 may be supplemented by giving the percentage of mice which, with these high doses, died within five minutes. It was approximately 70 per cent in Series 16 L and Series 13 L, but only 30 per cent in Series 11 L.

The doses *4x*, *1x*, *x/4*, and *x/16* produced a graded increase in survival times which was quite similar in the two separate experiments, Series 13 L and Series 11 L.

Morphology

Morphologic studies were limited to experiments with the highest doses, *16x* or *32x*.

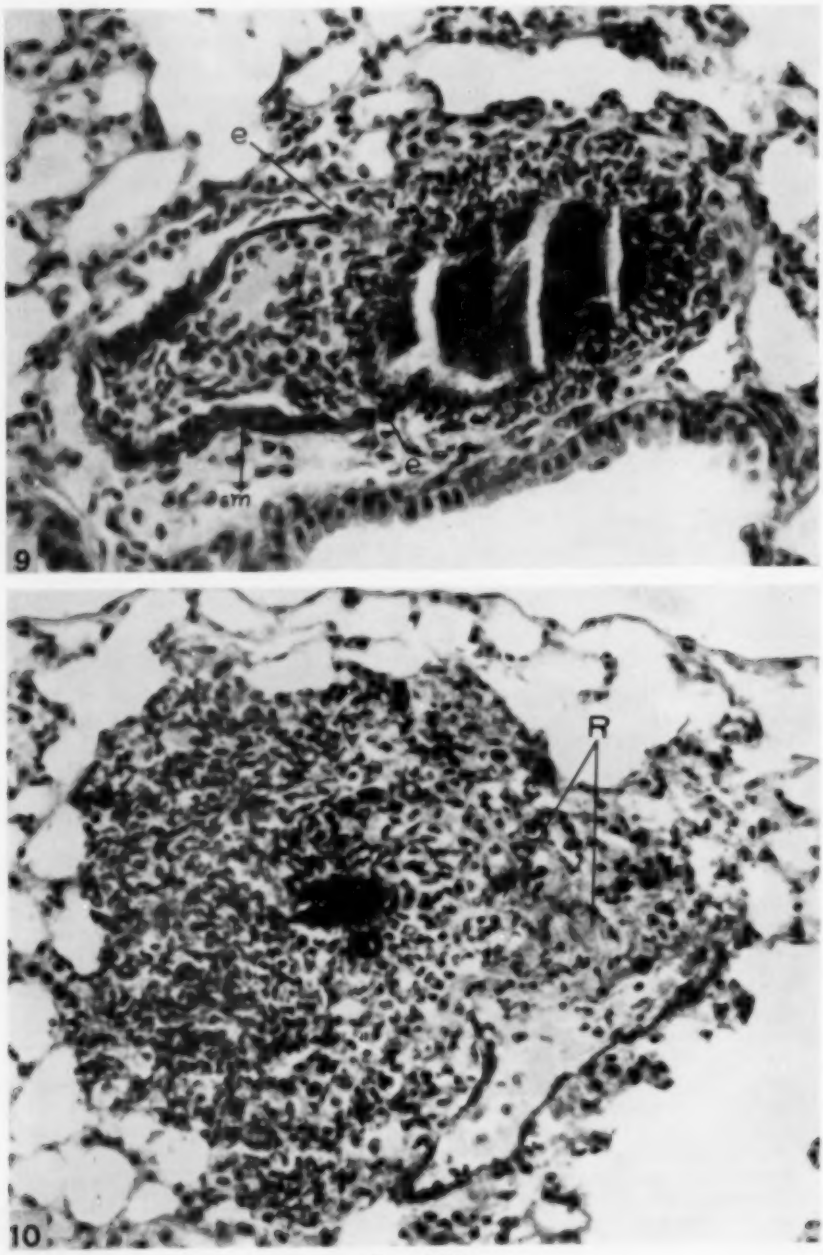
Morphology of Suspensions

The suspension with turbidity 2,600, which was used for injecting the dose *32x* into mice of Series 16 L, was examined by staining samples on a slide. The

FIG. 6. Series 10 L. Mouse 2; strain D4, fifteen minutes after injection of standard dose. Bacillary clump, 35 x 20μ in diameter, probably arrested in narrow segment of vessel. No intravascular reaction. *E*—elastica, *R*—erythrocytes. 3μ section, triple stain. (×400)

FIG. 7. Series 10 L. Mouse 4; strain D4, three hours after injection of standard dose. Embolic clump of bacilli (black), approximately 45 x 25μ in diameter, lodged in arteriole. Intensive intravascular reaction, mainly polymorphonuclear leukocytes. Places of wall marked by arrows seem to represent destruction of elastica rather than pre-existing gaps. 3μ section, triple stain. (×1,000)

FIG. 8. Branches of pulmonary artery, normal (Series 3 L, Mouse 2). Thick muscular wall (gray in the picture) in main branch *a*; alternation of presence and absence of muscle layer in branch *b*, and absence of muscle layer in branch *c*, without narrowing of bore. 6μ section, triple stain. (×100)



FIGS. 9–10

concentration of clumpy material in a drop proved to be so high that microscopic fields (at ×400 magnification) were either crowded by numerous medium-sized clumps or filled by one very large clump. Clumps of more than 200 x 100 μ in diameter were quite frequent, but it seems that such huge clumps are unstable since they were found neither in the standard suspension, made by dilution from the high concentrate, nor in the hearts or lungs of the injected mice.

Morphology of Lungs and Hearts

The lungs and hearts were examined in 5 of the mice which died within one minute after injection (dose *16x*, Series 11 L, Mouse 1; dose *32x*, Series 16 L, Mice 1, 2, 3, and 4). In each of the 5 mice, the lungs proved to be filled with large emboli, as seen in figure 22; as one would expect at such a short interval after injection, no intra- or extravascular reactions were visible. With respect to size, the emboli produced by *16x* or *32x* may be comparable to exceptionally large emboli observed with the dose *1x*. However, the number of embolized vessels and the total amount of embolic material in the different lobes after injection of *16x* or *32x* were always a multiple of those observed with the dose *1x*. This difference is illustrated in figures 5 and 22.

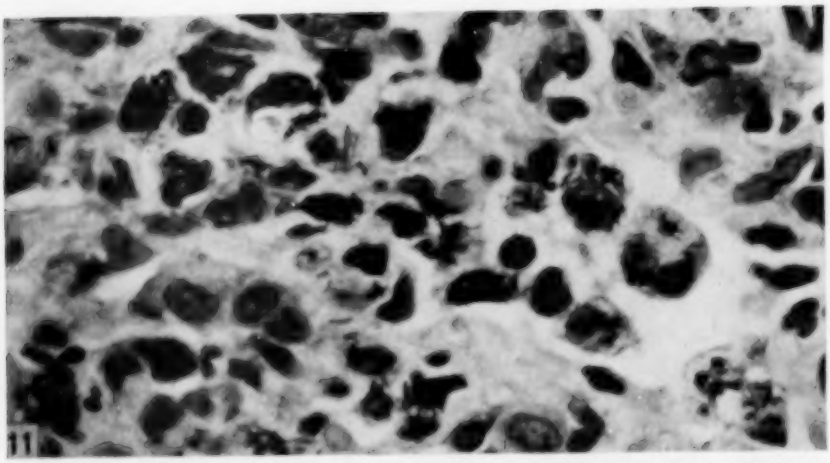
The hearts of several mice which died within a minute after injection showed clumps of bacilli mixed with the cardial blood. In addition, large clumps (up to 120 x 60 μ in diameter) were seen in myocardial vessels. This can be explained in two different ways. Either the clumps had travelled through the arteriovenous anastomoses of the lungs (4) or they had been forced into the coronary veins by excessive pressure in the right ventricle. As mentioned under *Survival*, of 20 mice of Series 16 L which had received the dose *32x*, 18 mice died immediately after injection, but 2 mice survived for three days. The amount of pulmonary embolic material in these 2 mice did not seem smaller than that in the 4 mice which died immediately. The only difference was that, during the three days of their survival, the 2 mice had produced reactive nodules around the bacillary clumps, resulting in 25 to 50 per cent consolidation of pulmonary tissue.

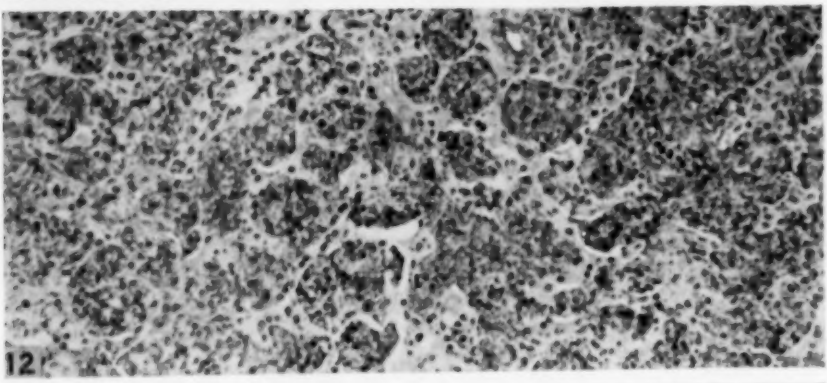
Discussion

Since the days of Borrel, the intravenous injection of clumpy suspensions of tubercle bacilli has been considered poor experimental technique. In his 1893 paper, Borrel (5) warned that clumps would cause pulmonary embolism and thus could "produce phenomena which have nothing to do with the tuberculous process." Many investigators after Borrel have mentioned well-dispersed suspen-

Fig. 9. Series 5 L, Mouse 1; strain D4, three hours after injection of standard dose. Intrapulmonary artery showing a wide gap where a large bacterial embolus (probably cracked during histologic procedures) and masses of reacting cells have broken through the wall; (*m*) muscular layer, (*e*) ends of ruptured elastica. 6μ section, triple stain. (×400)

Fig. 10. Series 3 L, Mouse 2; strain D4, twenty-four hours after injection of standard dose. Typical nodule of reaction around a bacterial clump which evidently has emerged from the ruptured vessel at the right lower part of the nodule. Extravascular erythrocytes (*R*) are remnants of small hemorrhage. 6μ section, triple stain. (×400)





Figs. 11–14

sions, small clumps, and large clumps, but very few attempts have been made to define these terms. The present paper is probably the first one in which clumps have been measured and suspensions characterized by microscopic pictures.

In the experiments reported here, the standard suspensions which were injected into tail veins of mice contained clumps of 20 x 10 μ and more in diameter. Bacterial emboli of corresponding size appeared in pulmonary arterioles. Yet, none of the feared circulatory disturbances took place, particularly no hemorrhagic infarctions.[6] There was a possibility that the rupture of embolized vessels, which was a regular occurrence, might cause hemorrhages and thereby obscure the reactions to the tubercle bacilli. However, hemorrhages were hardly noticeable; evidently the gap in the vascular wall was, at all times, well filled by the clump and the reacting cells.

In these circumstances, the obvious advantages of the embolic infection seemed to outweigh its more or less imaginary drawbacks.

The main advantages were: the fate of a substantial part of the inoculum, represented by the emboli, could be followed; readily demonstrable morphologic pictures were obtained which could be correlated with time after injection and reconstructed as a sequence of phases; the morphologic pictures and their time relations could be reproduced in a number of independent experiments.

The fate of intravenous inocula became an important problem with the increasing use of mice in experimental tuberculosis.[7] All workers who have investigated this aspect have stated that the bacilli are seen in the lungs some minutes or hours after injection, practically disappear from the lungs between the first and third days, and reappear in the lungs after approximately one week (3, 6, 7). Dr. H. Bloch informs us (7) that the aggregates found in his suspensions rarely contained more than 20 bacilli; this is equivalent to approximately 10 x 10 μ in diameter or less. Dr. G. T. Stewart wrote to us that, in the suspensions used

[6] Evidently, in the lungs of animals with a healthy circulation, extensive collaterals readily compensate for the obstruction of a number of arterioles and capillaries (*cf.* von Glahn and Hall (10)).

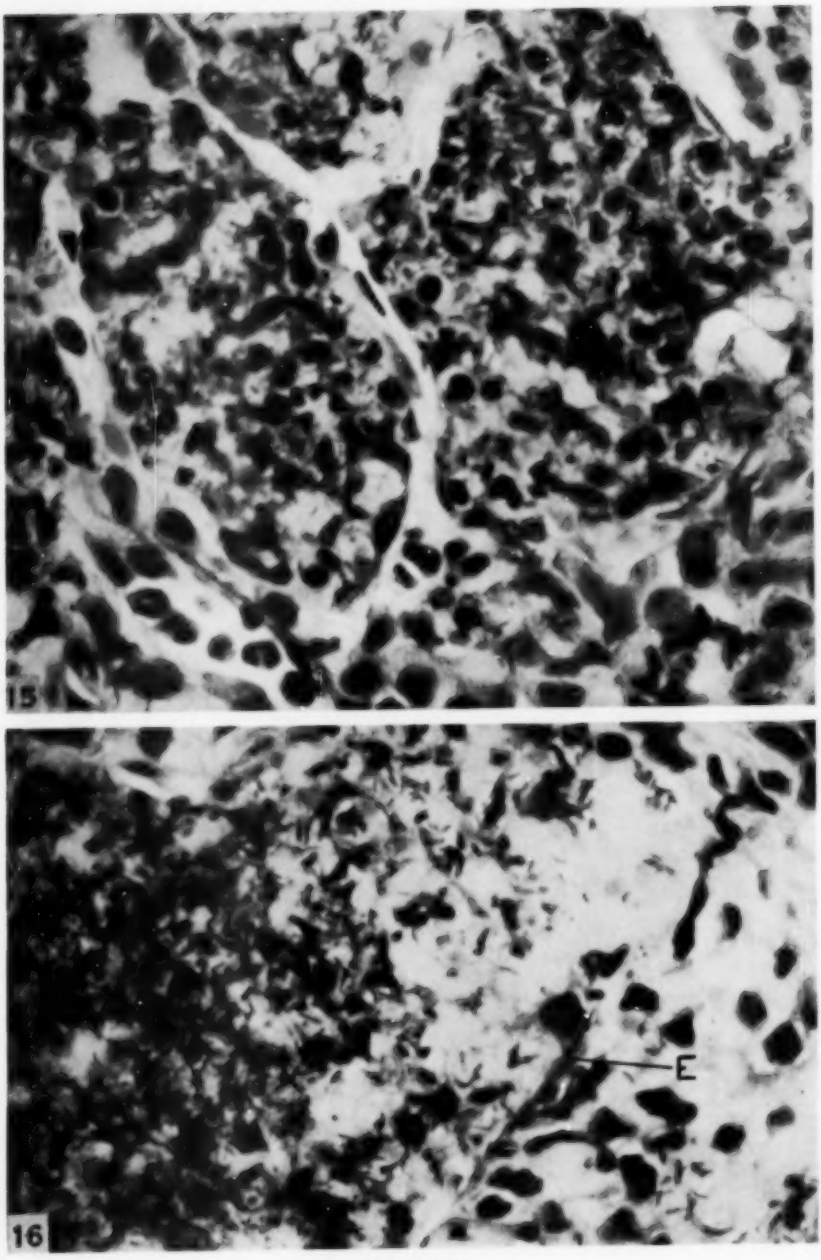
[7] Among the papers on experimental rabbit tuberculosis, the present writers found one with evidence of clumps and emboli of approximately 20 x 10μ: Wechsberg (11) showed pictures very similar to those of the present study, but he was not interested in the fate of the bacterial emboli.

FIG. 11. Series 20 L, Mouse 9; strain H37, four days after injection of standard dose. Mononuclear phagocytes laden with bacilli (clumps have disappeared). 3μ section, double stain. (×1,000)

FIG. 12. Series 20 L, Mouse 15; strain H37, fourteen days after injection of standard dose. Tuberculous pneumonia. 3μ section, double stain. (×200)

FIG. 13. Control mouse, approximately ten weeks old, sacrificed by illuminating gas. Normal expanded lungs, obtained by ligature of trachea before opening thoracic cavity; formalin fixation for twenty-four hours. (×3.5)

FIG. 14. Infected mouse, approximately ten weeks old; died fourteen days after injection of standard dose of strain D4. Extensive tuberculous consolidation, preventing collapse of lungs; volume similar to that of expanded normal lungs; formalin fixation. (×3.5)



FIGS. 15–16

in his 1950 paper (3), clumps of 20 x 10 μ in diameter may easily have occurred. It is beyond the scope of the present paper to discuss the possible fate of well-dispersed suspensions in the lungs of the mouse.[8] In the clumpy suspensions used, plenty of well-dispersed bacilli and small aggregates were also present, but it cannot be stated what happened to them after they reached the pulmonary capillaries. By focusing attention on clumps large enough to embolize arterioles, it was possible to observe the different stages of the process by which an *originally intravascular mass of bacilli leaves the vessel and moves into the lung tissue proper.* This transition could not be demonstrated with other methods. By what forces the wall of the embolized arteriole is ruptured is a matter for speculation. Only the thin-walled segment of vessels (figure 8) can be perforated within a few hours, as Dr. A. R. Rich has pointed out to the present writers.[9]

Subsequently, the escaped embolus forms the center of a reactive nodule until dispersion by mononuclear phagocytes terminates the history of a clump.

The reconstruction of the process was not difficult because there could be only one sequence of the phases observed. Moreover, the different phases occurred at definite intervals after injection, with moderate overlappings between different mice or between different areas in the lungs of one mouse. The only instance in which a phenomenon seemed to occur at an entirely "wrong" time was the preservation of a large intravascular clump as late as twelve days after injection (table 2, 12 days, Series 16 L). A reasonable explanation can be derived from the photomicrograph (figure 21). Probably, the large clump was arrested in the thick-walled part of an artery; therefore, the intravascular cellular reaction (which is still visible) could not rupture the wall. Later on, the pneumonic and necrotizing processes in the lung encircled the artery and dissolved its muscular layer, while fragments of the elastica persisted. Under these conditions the clump could not be reached and dispersed by mononuclear phagocytes.

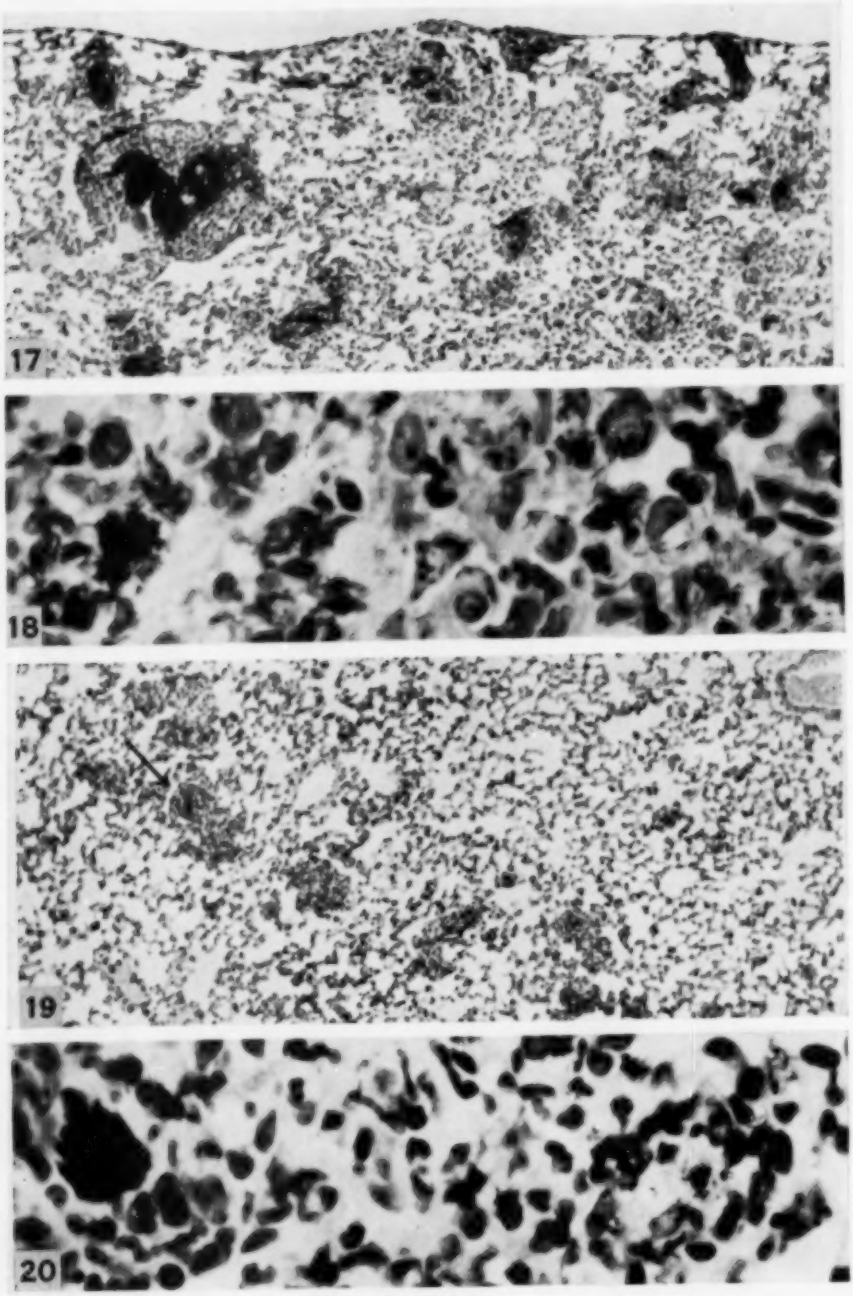
The satisfactory reproducibility of the morphologic pictures and of their time relations evidently derives from the reproducibility of the routine procedure of the Bacteriology Laboratory. The results of the survival tests, which were made in parallel with the morphologic studies, strongly suggested that the mice

[8] It is not surprising that well-dispersed bacilli, although delayed in the tortuous pulmonary capillaries, are gradually washed out by the bloodstream. The problem is their reappearance after some time and subsequent persistence in the lungs.

[9] Interesting analogies are found in the paper by von Glahn and Hall (10), who described how sterile cotton emboli and resulting granulomas moved through the walls of pulmonary arteries in rats. Their illustrations show that the ruptures occurred in the thin-walled (nonmuscular) parts of the vessels.

FIG. 15. Series 10 L, Mouse 16; strain D4, twelve days after injection of standard dose. High magnification of a pneumonic area (pneumonic plugs) showing two alveoli with well-recognizable outlines, numerous bacilli, and necrosis of exudate cells. 3μ section, triple stain. (×1,000)

FIG. 16. Same section as in figure 15. This area shows destruction of alveolar pattern by massive necrosis. Large numbers of bacilli on left side of picture, remnants of elastic fibers (*E*) on right side. (×1,000)



FIGS. 17–20

sacrificed for morphologic studies were in all respects equivalent to those used in the routine survival tests.

It is difficult, however, to establish any relation between the clumpiness of suspensions and survival time. Bloch (8) has made an attempt to correlate survival times with the state of dispersion of suspensions. The "clumps" in his table III were much smaller than 20 μ in diameter (personal communication by Dr. Bloch). Well-dispersed suspensions and small, loose clumps can be characterized by the approximate number of bacilli per unit (Bloch's table I), but large, densely packed clumps, as used in the present study, are best defined by their diameters in micra. It would be useless to compare well-dispersed and clumpy suspensions on the basis of dry or wet weights. Besides these technical difficulties, it seems unlikely that there should be any *direct* relation between clumpiness of suspensions and survival times. The primary inoculation of the lungs with large clumps, on the one hand, or with well-dispersed bacilli, on the other hand, initiates two entirely different processes, although both finally converge to the same picture of fatal tuberculous pneumonia. If very high doses of the clumpy suspensions (16x or 32x) were injected, massive pulmonary embolism caused immediate death of the mice. Youmans and Youmans (9) have also produced immediate death of mice by injecting very high doses (10 or 12 mg.). Before discussing their hypothesis of possible toxic death, it would be instructive to see microscopic pictures of their suspensions.

It has been difficult to compare the results of the present study with those found in the literature, mainly because of lack of morphologic data concerning the suspensions used by other investigators. In addition to information on preparation of suspensions, turbidity values, and volume injected, future publications should include microscopic pictures of suspensions, numbers of tubercle bacilli for small and loose aggregates, and diameters (in micra) for dense or large clumps.

Summary

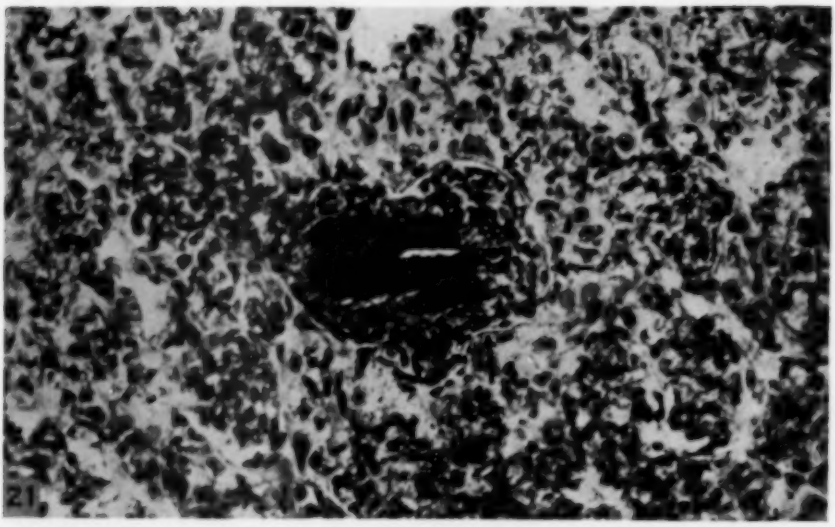
Morphologic studies have been made of an experimental mouse tuberculosis which is used in routine survival tests for drug screening and evaluation.

FIG. 17. Series 20 L, Mouse 6; strain H37, twenty-four hours after injection of standard dose. Maximum of embolic material in lungs observed with this dose: very large clumps in this and other areas of the section, and in other lobes. 3μ section, double stain. (×100)

FIG. 18. Same section as figure 17, but different area and higher magnification. This area shows scattered bacilli and small aggregates, many of them in mononuclear phagocytes. (×1,000)

FIG. 19. Series 20 L, Mouse 5; strain H37, twenty-four hours after injection of standard dose. Minimum of embolic material in lungs: one small clump (14 x 12μ) in the whole section, marked with arrow; clumps were equally small and scarce in other lobes. Tiny aggregates of bacilli (shown in figure 20) have caused the numerous nodules of reaction. 3μ section, double stain. (×100) *Note*: Mouse 5 and Mouse 6 of Series 20 L received each 0.2 ml. of the same charge of one syringe.

FIG. 20. Same section and area as in figure 19, at higher magnification. In addition to the clump of figure 19, which is near the left margin of the present picture, there are scattered bacilli and small aggregates, mostly in mononuclear phagocytes. (×1,000)



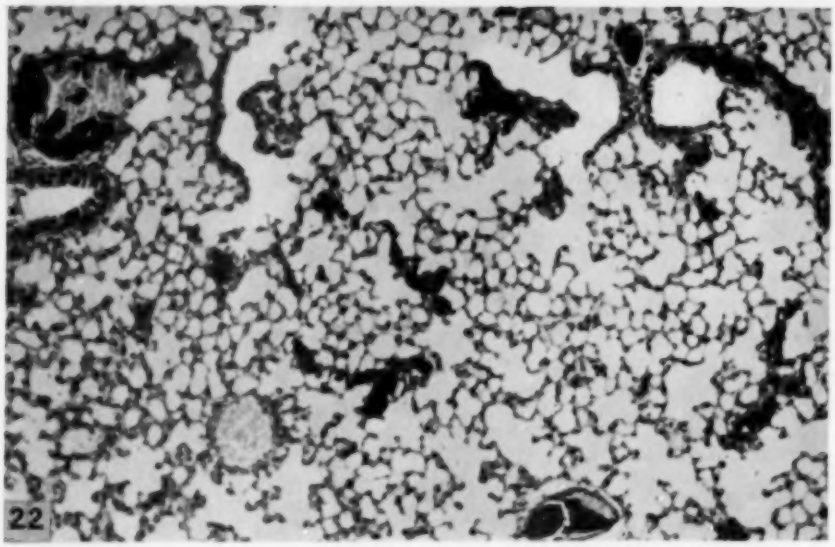


FIG. 21. Series 10 L, Mouse 16; strain D4, twelve days after injection; same mouse as in figures 15 and 16. Unusual preservation of a large intravascular clump in midst of area of pneumonia and massive necrosis; remnants of vascular elastica marked by arrows. For interpretation, *see* text. 3μ section, triple stain. (×400)

FIG. 22. Series 16 L, Mouse 3; strain D4, thirty-two times the standard dose; death ten seconds after injection. Lung showing very large embolic masses which caused death mechanically; no tissue reaction. 6μ section, double stain. (×100) *Note*: All areas of all lobes showed similar amounts of embolic masses.

TABLE 2

HISTOLOGY OF LUNGS OF 71 MICE, FROM FIVE MINUTES TO FOURTEEN DAYS AFTER INTRAVENOUS INJECTION OF STANDARD DOSE OF TUBERCLE BACILLI

Strain H37 was used in Series 20 (marked with asterisk), while strain D4 was used in the 6 other series.

TIME AFTER INJECTION	SERIES	NUMBER OF MICE	LARGE CLUMPS† OF TUBERCLE BACILLI	INTRAVASCULAR REACTION	RUPTURE OF VASCULAR ELASTICA	EXTRAVASCULAR NODULES	PHAGOCYTES WITH BACILLI	PNEUMONIC PLUGS	MASSIVE NECROSIS	CONSOLIDATED AREA, ESTIMATED AS PER CENT	
5 minutes	10 L	1	+	−	−	−	−	−	−	0	
	11 L	2	++	−−		−−	−−	−−	−−	0	0
	16 L	2	++	−−		−−	−−	−−	−−	0	0
	*20 L	2	++	−−		−−	−−	−−	−−	0	0
15 minutes	10 L	1	+	±	±	−	−	−	−	0	
1 hour	10 L	1	+	+	+	−	−	−	−	0	
	*20 L	2	++	++		−−	−−	−−	−−	0	0
3 hours	5 L	2	++	++	++	−−	−−	−−	−−	0	0
	10 L	1	+	+	+	−	−	−	−	0	
6 hours	5 L	2	++	++	+	−−	−−	−−	−−	0	0
	10 L	1	+	+		−	−	−	−	0	
12 hours	5 L	2	++	++		++	−−	−−	−−	0	0
24 hours	3 L	2	++	++	+	++	−−	−−	−−	1–25	1–25
	5 L	2	++	++	+	++	−−	−−	−−	1–25	1–25
	10 L	1	+	+		+	±	−	−	1–25	
	11 L	2	++	++		++	+−	−−	−−	1–25	1–25
	16 L	2	++	++	+	++	±−	−−	−−	1–25	1–25
	*20 L	2	±+	++		++	++	−−	−−	1–25	1–25
2 days	10 L	1	+	−		+	+	−	−	1–25	
	16 L	2	±±	−−		±±	−−	−−	−−	1–25	1–25
	*20 L	2	−−	−−		++	++	−−	−−	1–25	1–25
3 days	16 L	2	±±	−−		++	++	−−	−−	1–25	1–25
4 days	3 L	2	++	+−		++	++	−−	−−	25–50	25–50
	10 L	1	+	−		+	+	−	−	25–50	
	16 L	2	−−	−−		++	++	−−	−−	1–25	1–25
	*20 L	2	−−	−−		++	++	−−	−−	1–25	1–25
6 days	3 L	2	−±	−−		++	++	−−	−−	1–25	25–50
	10 L	1	−	−		+	+	−	−	25–50	
8 days	3 L	2	−−			++	++	+−	−−	25–50	25–50
	10 L	1	±‡			+	+	+	+	>50	
	*20 L	2	−−	−−		++	++	−−	−−	25–50	25–50
9 days	3 L	2	−−			++	++	++	−−	1–25	25–50
11 days	3 L	2	−−			++	++	++	−−	25–50	1–25
	10 L	1	−			−	+	+	+	>50	
	11 L	2	−−	++		−−	±±	++	±±	25–50	25–50
	*20 L	2	−−	−−		++	++	++	−−	25–50	25–50
12 days	8 L	2	−−			−−	++	++	++	>50	>50
	8 L	2	−−			−−	++	++	++	>50	>50
	10 L	1	+§	+§		−	+	+	+	>50	
13 days	3 L	2	−−			−+	++	++	++	>50	>50
14 days	8 L	1	−			−	+	+	+	>50	
	*20 L	2	−−	−−		−−	++	++	++	>50	>50

† Clumps of at least 20 x 10μ in diameter.

‡ One clump.

§ One large clump in remnant of vessel (*see* figure 21).

Explanation of symbols: + present, ± doubtful, − absent, blank not examined. If 2 symbols are entered, each refers to one mouse.

TABLE 3

EFFECT OF DIFFERENT DOSES OF STRAIN D4 ON SURVIVAL TIME OF MICE (INFECTED IN PARALLEL WITH MICE INFECTED AND SACRIFICED FOR MORPHOLOGIC STUDY)

DATE OF INFECTION (SERIES NUMBER)	DOSE	NUMBER OF MICE*	SURVIVAL TIMES			
			Actual Range	Calculated Values†		
				16 Per Cent Dead	50 Per Cent Dead	84 Per Cent Dead
September 12, 1951 (Series 13 L Contr.)	16x	20	30 seconds–20 hrs.	30 seconds*c*	30 seconds*c*	20 hours*c*
	4x	20	3 days–9 days	3 days	5 days	8 days
	1x	20‡	8–18 days	10 days	12 days	15 days
	x/4	20	14–22 days	14 days	16 days	18 days
	x/16	20	16–59 days	17 days	28 days	47 days
October 18, 1951 (Series 11 L Contr.)	16x	20	10 seconds–9 days	10 seconds*c*	2 days*c*	6 days*c*
	4x	20	2 days–13 days	4 days	7 days	12 days
	1x	10‡	9–17 days	10 days	13 days	17 days
	x/4	20§	13–>54 days	14 days	17 days	20 days
	x/16	10‖	16–>54 days	17 days	24 days	34 days
February 11, 1952 (Series 16 L Contr.)	32x	20	10 seconds–3 days	10 seconds*c*	10 seconds*c*	10 seconds*c*
	1x	10‡	9 days–17 days	11 days	13 days	15 days

* At termination of experiments, all mice had died except in groups marked § and ‖.

† Calculated according to Litchfield's method (2) except for entries marked *c*, which are actual values.

‡ Identical with mice of Series 13 L, 11 L, and 16 L in table 1.

§ Two mice which were sacrificed after 54 days (termination of experiment) were not included in the calculated values.

‖ Two mice were excluded from the calculated values: one was sacrificed after 28 days for morphologic study, the other was sacrificed after 54 days (termination of experiment).

In the lungs of the mice, bacillary emboli 20 x 10 μ in diameter or larger were found immediately after injection; these emboli originated from clumps of similar size which were present in the suspensions of standard concentration. The emboli did not cause hemorrhagic infarctions of the lungs.

In contrast to the known disappearance of well-dispersed suspensions from the lungs, the retention of clumpy suspensions in the lungs made it possible to follow the fate of a substantial part of the inoculum, namely, the embolized clumps.

It was possible to reconstruct the sequence of events from the intravascular reaction around the emboli to break-through into the pulmonary tissue with subsequent dispersion of bacilli by mononuclear phagocytes, resulting in fatal tuberculous pneumonia.

Organs other than the lungs showed a relatively slight tuberculous involvement.

The morphologic observations were similar with injections of strain D4 and strain H37Rv.

Different doses of strain D4, varying from thirty-two times the standard dose to one-sixteenth of the standard dose, produced a graded increase in survival time. With the standard dose, 50 per cent of the mice survived approximately fourteen days after injection. The highest doses caused immediate death by massive embolism. With one-sixteenth of the standard dose, 50 per cent survived approximately twenty-six days.

The morphologic pictures and their time relations could be reproduced in a number of experiments spaced over two years. Survival tests made in parallel with the morphologic studies suggested that the mice sacrificed for morphologic studies were in all respects equivalent to those used in routine survival tests.

Sumario

Tuberculosis Pulmonar Embólica Experimental en el Ratón

Estos estudios morfológicos versaron sobre una tuberculosis murina experimental que se emplea en ensayos corrientes de sobrevivencia para la selección y justipreciación de drogas.

En los pulmones de los ratones, descubriéronse inmediatamente después de la inyección émbolos bacilares de 20 por 10 μ o más de diámetro; esos émbolos tenían su origen en grumos de tamaño semejante que se encontraban en las suspensiones de concentración tipo. Los émbolos no provocaron infartos hemorrágicos de los pulmones.

En contraposición a la conocida desaparición de los suspensiones bien dispersadas de los pulmones, la retención de las suspensiones grumosas en los pulmones permitió observar el destino de una parte substancial del inóculo, o sea, de los grumos embolizados.

Fué posible reconstruir así la evolución de los acontecimientos desde la reacción intravascular alrededor de los émbolos hasta la irrupción en el tejido pulmonar con la subsiguiente dispersión de los bacilos por fagocitos mononucleares, y neumonía tuberculosa letal.

Aparte de los pulmones, los demás órganos revelaron una invasión tuberculosa relativamente leve.

Las observaciones morfológicas fueron similares con las inyecciones de la cepa D4 y la cepa H37Rv.

Distintas dosis de la cepa D4, que variaron de treinta y dos veces la dosis tipo a un dieciseisavo de ésta, produjeron un aumento gradual en el tiempo de sobrevivencia. Con la dosis tipo, 50 por ciento de los ratones sobrevivieron aproximadamente catorce días después de la inyección. Las dosis máximas ocasionaron la muerte inmediata por embolia masiva. Con un dieciseisavo de la dosis tipo, 50 por ciento sobrevivieron unos veintiséis días.

En varios experimentos espaciados a través de dos años, fué posible reproducir los cuadros morfológicos y sus relaciones cronológicas. Las pruebas de sobrevivencia, ejecutadas paralelamente a los estudios morfológicos, sugerían que los ratones sacrificados para los estudios morfológicos eran en todos sentidos equivalentes a los usados en los ensayos corrientes de sobrevivencia.

Resume

Tuberculose pulmonaire embolique expérimentale chez la souris

Des recherches morphologiques ont été effectuées sur la tuberculose expérimentale de la souris utilisée pour les tests habituels permettant d'établir les taux de survie pour la sélection et l'évaluation des drogues.

Des embolies bacillaires d'un diamètre de 20/10μ, ou plus, ont été découvertes immédiatement après les injections de suspensions de concentration standard; ces embolies avaient pour origine des agglomérats de même volume que ceux présents dans les suspensions. Ces embolies ne déterminaient pas d'infarctus hémorragiques dans le poumon.

Contrastant avec la disparition des suspensions bien dispersées sans agglomérats, au niveau des poumons, la rétention pulmonaire des suspensions avec agglomérats a permis de suivre l'évolution d'une partie substantielle de l'inoculum, c'est-à-dire de l'agglomérat embolisé.

La suite des évènements pouvait être observée à partir de la réaction intravasculaire au niveau de l'embolus jusqu'à son passage dans le tissu pulmonaire avec dispersion subséquente des bacilles par les phagocytes mononucléaires et, enfin, jusqu'à la pneumonie tuberculeuse fatale.

En dehors du poumon, d'autres organes ont présenté des modestes lésions tuberculeuses.

Les observations morphologiques effectuées avec l'injection des souches D4 et H37Rv étaient identiques.

Des augmentations progressives du temps de survie ont été observées avec différentes doses de la souche D4 qui s'échelonnaient entre 32 fois la dose standard et un seizième de celle-ci.

Avec la dose standard, 50% des souris survivaient environ quatorze jours après l'injection. Les doses les plus élevées provoquaient la mort immédiate par embolie massive. Un-seizième de la dose standard entrainait une survie d'environ vingt-six jours chez 50% des souris.

Les aspects morphologiques et leurs corrélations dans le temps ont été reproduits au cours d'expérimentations espacées sur deux années. Les tests de survie, effectués parallèlement aux études morphologiques, laissent supposer que les souris sacrifiées pour cette étude morphologique ne différaient point de celles utilisées dans les tests habituels de survie.

Acknowledgment

We wish to thank Dr. H. J. White of these laboratories for his cooperation and continuous advice in all phases of this investigation. We are indebted to Misses Irene A. Salamandra, Dorothy J. Cecire, and Elly Heyder, also of these laboratories, for help in various parts of this paper.

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Case Reports

OBSERVATIONS ON PYRAZINAMIDE (ALDINAMIDE) IN PULMONARY TUBERCULOSIS[1]

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INTRODUCTION

The present study was an attempt to confirm the experience of Yeager and associates who found pyrazinamide (Aldinamide)[2] effective in the treatment of tuberculosis. Pyrazinamide was experimentally synthesized by Kushner and his associates (1). Initial studies by Malone and co-workers (2) revealed that pyrazinamide given orally or subcutaneously was effective in the treatment of experimental tuberculosis in mice. Dessau and associates (3), using guinea pigs, found a moderate beneficial effect which could be increased by the concomitant administration of either streptomycin or para-aminosalicylic acid (PAS).

Yeager, Munroe, and Dessau (4) treated 43 patients with human pulmonary tuberculosis for periods between three and seventy-five days. They noted that pyrazinamide produced uniform clinical improvement. Fever disappeared, cough and expectoration became noticeably reduced, and the patients reported considerable subjective improvement. In 6 of their 25 patients with far advanced tuberculosis and in 12 of 15 with moderately advanced tuberculosis, some degree of roentgenographic improvement occurred. Of 33 patients with sputum positive for tubercle bacilli prior to therapy, 10 developed sputum negative for tubercle bacilli on microscopic examination, and also on culture in 4 patients. There developed an appreciable degree of pyrazinamide resistance in the infection of all of the 12 patients whose bacilli were tested before and after therapy. Toxic effects of the drug were manifested commonly by joint pains without redness or swelling and, occasionally, by a febrile reaction. Two patients developed jaundice and abnormal liver function tests suggestive of parenchymal liver involvement, which slowly improved, leaving no residuals of hepatic dysfunction six to eight months after completion of therapy. A dose of 700 mg. four times daily for forty-two days was recommended by these investigators.

PLAN OF INVESTIGATION

In the present study, 29 patients were selected for treatment, 11 with pyrazinamide alone and 18 with pyrazinamide plus other agents.

Of the 11 patients who received pyrazinamide alone, 6 represented patients consecutively admitted to the service who had had no previous chemotherapy at all. These were placed on a projected course of six weeks of pyrazinamide, 750 mg. four times daily. The

[1] From the Pulmonary Disease Section, Medical Service, Veterans Administration Medical Teaching Group Hospital, Memphis, Tennessee.

[2] The pyrazinamide (Aldinamide) was kindly supplied for the purpose of this study by Lederle Laboratories, American Cyanamid Company, Pearl River, New York.

purpose was to study the effect of pyrazinamide in the average, presumably favorable, case of pulmonary tuberculosis when given for the length of time recommended by Yeager and his associates. In view of other proved effective forms of therapy available for these patients, there was hesitancy in proceeding beyond this period of time. The remaining 5 patients in the group had less favorable disease which had failed to improve on previous antimicrobial therapy with other agents and which was associated with organisms which were streptomycin resistant. In these patients, it was considered justifiable to project where feasible a longer course of treatment (up to three months) with pyrazinamide, 750 mg. four times daily.

The 11 patients who received pyrazinamide alone consisted of 5 white and 6 Negro males. Three patients were in the third decade of life, 3 in the fourth, 3 in the fifth, and 2 in the sixth decade of life. At the start of therapy, 9 patients had progressive pulmonary tuberculosis and, in 2, the disease was stationary. Seven patients had far advanced tuberculosis, and 4 had moderately advanced disease. All except one had sputum positive for tubercle bacilli at the time therapy was begun. The exception was a patient whose sputum cultures had been positive for tubercle bacilli but became negative (unknown at the time to the investigators) just before therapy was started. In 6 patients the organisms were completely susceptible to streptomycin, and in 4 they were resistant. Drug susceptibility was not determinable in the patient with the "negative sputum," but he had had two prior courses of streptomycin and PAS therapy, and the organisms may well have been resistant.

OBSERVATIONS

Pyrazinamide Alone

In 7 of the 11 patients who received pyrazinamide alone, the treatment was followed by obvious clinical improvement, manifested by a decrease in cough and expectoration, better appetite, and a moderate to marked sense of well-being. As would be expected, the results were better in the original treatment group than in the group which had received previous antituberculous chemotherapy (re-treatment group). Five of the 6 in the original treatment group improved clinically, and all showed weight gain. Two of the 5 patients in the re-treatment group (table 1) improved clinically, but none showed weight gain. In 2 patients in the re-treatment group, the disease remained unchanged, while in one patient in each group the lesions became worse. Both of the latter patients showed initial clinical improvement but later regressed while still under therapy.

The changes visible in the chest roentgenograms showed to less advantage. In the

TABLE 1

PATIENTS RE-TREATED WITH PYRAZINAMIDE ALONE

PATIENT	STREPTOMYCIN-SUSCEPTIBILITY OF ORGANISMS	CLINICAL CHANGE	SPUTUM "CONVERSION"	ROENTGENOGRAPHIC CHANGE	LENGTH OF TREATMENT (WEEKS)
1. A.C.	R	4	No	4	3
2. C.M.	R	2	No	6	13
3. J.C.	R	5	No	4	13
4. C.G.	R	2	No	4	13
5. P.M.*	?	4	?	4	6

* Patient's sputum culture became negative for *M. tuberculosis* just prior to start of therapy.

4 = no change; 3, 2, 1 = slight, moderate, and marked improvement, respectively; 5, 6, 7 = slight, moderate, and marked worsening, respectively.

R = resistant

original treatment group of 6, one patient showed moderate roentgenographic improvement and 2 showed slight improvement. In the re-treatment group of 5, none showed roentgenographic improvement. In one case in each group, in the patients who had shown poor clinical responses, there was roentgenographic worsening. The sputum did not become negative for tubercle bacilli on culture in any of the patients, but in 2 patients of the original treatment group it became negative on microscopic examination.

Individual case reports of the 6 original treatment patients are briefly summarized.

I. H., a 54-year-old white male, was admitted on June 10, 1953, with a history of pulmonary tuberculosis since 1945, considered arrested since 1946, who developed symptoms of reactivation in March, 1953. He had fever up to 100°F. Fine moist rales were found at the right apex. The chest roentgenograms showed moderately advanced disease with a progressive productive infiltrate in the right upper lung. The sputum was positive for tubercle bacilli. Liver function tests were normal.

With pyrazinamide, 750 mg. four times daily, the patient became afebrile. There was a decrease in cough and expectoration, increased appetite, and a 4 pound weight gain. At the end of three weeks, the chest roentgenogram showed slight clearing, but the sputum remained positive for acid-fast bacilli on microscopic examination. However, the patient became jaundiced. The total serum bilirubin rose to 18 mg. per 100 ml.; on direct test, 11.9 mg. per 100 ml. The thymol turbidity test showed 9.1 cc. of $BaSO_4$, the cephalin flocculation test was 3 plus, and the urine was positive for bilirubin. Therapy with pyrazinamide was discontinued.

Although the jaundice was believed to be due to a toxic hepatitis, the patient had been subjected to blood studies four months previously and, since he also had an elevated leukocyte count and lymphocytosis, neither homologous serum jaundice nor infectious hepatitis could be excluded. On discontinuation of the pyrazinamide, the patient began to improve and, four weeks later, his liver function studies were approaching normal values.

K. C. C., a 25-year-old Negro male, was admitted on June 25, 1953, with a two-year history of symptoms suggestive of pulmonary tuberculosis. His temperature was 102.4°F., he had night sweats, and he had lost 39 pounds in weight. He had severe cough and expectorated 480 cc. daily of thick foul purulent sputum. The chest roentgenogram showed far advanced disease with a dense lesion extending from the right apex to the third anterior interspace and containing a radiolucency 5 cm. in diameter. The sputum was positive for tubercle bacilli. On July 2, 1953, therapy with pyrazinamide, 750 mg. four times daily, was started. The patient became afebrile within five days, the cough decreased, the sputum declined to 120 cc. daily and became less purulent. His appetite improved, and he gained 7 pounds in weight.

The sputum remained positive for acid-fast bacilli, although fewer were found, and no significant change was seen in the chest roentgenogram at the conclusion of the therapy.

J. H. M., a 52-year-old white male, was admitted on June 27, 1953, complaining of dyspnea on exertion for three years and subsequent orthopnea and paroxysmal nocturnal dyspnea. He also complained of a productive cough, fever, and weight loss. Pedal edema was noticed four days before admission. On examination he showed evidence of pulmonary emphysema, chronic bronchitis, cor pulmonale with congestive failure, and pulmonary tuberculosis. The chest roentgenogram showed far advanced disease, with dense patchy infiltrates throughout both lung fields and small areas of radiolucency. The sputum was positive for tubercle bacilli. The myocardial insufficiency responded to salt restriction, Mercuhydrin, and digitoxin. With pyrazinamide, 750 mg. four times daily, there was improvement of cough, a 50 per cent decline in sputum, and a reduction of fever.

Approximately three weeks after the start of pyrazinamide therapy, the symptoms suddenly became aggravated and the chest roentgenogram showed increased disease. The sputum was still positive for tubercle bacilli. Therapy was changed to a combination of streptomycin, PAS, and isoniazid, with prompt clinical improvement.

P. K., a 43-year-old Negro male, was admitted on June 29, 1953, with a six-month history compatible with pulmonary tuberculosis. The chest roentgenogram showed moderately advanced disease with mottled infiltrates from the left apex to the fourth anterior interspace and containing a 1 cm. radiolucency. Therapy with pyrazinamide, 750 mg. four times daily, for six weeks caused a decrease in cough and sputum, with increased appetite and a weight gain of 8 pounds. Practically all of the clinical improvement occurred within the first four weeks.

The sputum became negative for acid-fast bacilli on microscopic examination but remained positive for *M. tuberculosis* on culture. There was no significant change in the chest roentgenogram.

R. D. S., a 30-year-old white male, entered the hospital on June 23, 1953, with a six-month history of symptoms suggestive of tuberculosis. The chest roentgenogram showed moderately advanced disease, with mottled infiltrates extending from the right apex to the level of the second anterior interspace. Sputum was positive for acid-fast bacilli on microscopic examination. Therapy with pyrazinamide, 750 mg. four times daily, for six weeks caused a slight decrease in the small amount of sputum but no change in the slight cough.

His appetite improved, and he gained 11 pounds in weight. The chest roentgenogram revealed slight clearing of the abnormal shadows, but the sputum remained positive for tubercle bacilli.

E. A., a 44-year-old white male with a history of pulmonary tuberculosis since 1948, had been on leave from a sanatorium since April, 1953. He had been treated from 1948 to April, 1953, with bed rest only. He was admitted to this hospital on July 17, 1953, because a re-examination revealed sputum positive for tubercle bacilli. The chest roentgenogram showed patchy densities throughout the upper one-third of both lungs. The sputum was again found positive for tubercle bacilli. The patient was given six weeks' therapy with pyrazinamide, 750 mg. four times daily. His cough disappeared, his appetite improved, and he gained 10 pounds.

The sputum became negative for acid-fast bacilli on microscopic examination. The chest roentgenograms showed moderate clearing of the abnormal shadows.

Comment: These findings signified to the present writers that pyrazinamide can exert definite favorable influence on patients with pulmonary tuberculosis, as demonstrated by a decrease in cough and expectoration, an improved sense of well-being and, in the more favorable cases, by weight gain. Although the roentgenographic and bacteriologic changes were not impressive, even in the favorable cases, yet, if the short time of therapy is considered (merely six weeks), then the response here, too, appeared more satisfactory. In the re-treatment group, the benefit of pyrazinamide appeared slight, but these cases were failures of prior therapy with streptomycin and PAS, which are proved and, presumably, more potent agents. The conclusion of Yeager and associates that pyrazinamide was effective for only a short period of time appeared to be borne out by *patient J. H. M.*, who suddenly "escaped" from the drug's beneficial influence after only three weeks of therapy. *Patient I. H.*, with probable toxic hepatitis due to pyrazinamide, seemed to confirm Yeager's findings with regard to the drug's potential hepatotoxic properties.

Pyrazinamide Administered Concurrently with Other Antituberculous Drugs

In view of the experimental evidence obtained by Dessau and co-workers (3) that the beneficial effect of pyrazinamide was enhanced in guinea pigs by the concomitant administration of either streptomycin or PAS, the present writers were curious to know if such or similar combinations would prove beneficial in human pulmonary tuberculosis.

For this purpose 18 patients were selected, consisting of 13 white males, 4 Negro males, and one white female. Three patients were in the third decade of life, 5 in the fourth, 4 in the fifth, and 6 in the sixth. Nine patients had progressive pulmonary tuberculosis; in 7 the tuberculosis was stationary; and in 2 it was regressive. All had sputum positive for tubercle bacilli before therapy. In 11 patients, the bacilli were susceptible to streptomycin, and in 7 the bacilli were resistant. With the pyrazinamide, 10 patients received streptomycin or dihydrostreptomycin, one received PAS, 3 patients received streptomycin and PAS, and 4 received isoniazid (table 2).

Clinical course: There was apparent clinical improvement in 10 patients, no change in 6, and worsening in 2 patients. The 2 patients who showed worsening, however, had severe complicating disease not related to the tuberculosis. One died during the course of therapy from renal infection secondary to paraplegia, and the other died of lymphosarcoma. Clinical improvement occurred in 8 of the 11 patients whose tubercle bacilli were still susceptible to streptomycin. In only 2 of 7 patients was there clinical improvement when the organisms were streptomycin resistant; and, in one of these, isoniazid was given with the pyrazinamide. In fact, all of the 4 patients who received isoniazid in addition to the pyrazinamide showed some clinical improvement.

Roentgenographic course: Again the patients showed less improvement roentgenographically than clinically. Eight of the 18 patients showed roentgenographic improvement; in 3, it was moderate; in the remaining 5, slight. Seven of these 8 patients had streptomycin-susceptible bacilli. The only patient with streptomycin-resistant organisms who showed roentgenographic improvement was the one who had received concurrent isoniazid. Again, all of the 4 patients who received concurrent isoniazid therapy showed roentgenographic improvement. In one, the improvement was moderate; in 3, it was slight. Four patients showed worsening roentgenographically. Two of these had streptomycin-resistant organisms; the other 2 had streptomycin-susceptible organisms.

Bacteriologic findings: The sputum became negative for tubercle bacilli on culture in 5 of the 18 patients who received pyrazinamide together with other chemotherapy. All except one of the 5 patients had had streptomycin-susceptible bacilli. Two of these patients received concurrent isoniazid while the others received streptomycin or dihydrostreptomycin. All of the patients whose sputum "converted" also showed improvement clinically and roentgenographically.

Susceptibility studies to pyrazinamide were performed by Tarshis and Weed (5) of the Tuberculosis Research Laboratory of this unit, using solid media. In all tests performed, even with organisms before therapy, no inhibition could be shown on American Trudeau Society medium and only partial inhibition on blood medium and on Herrold medium containing 100 γ and 1,000 γ of pyrazinamide per ml.

It has been suggested that pyrazinamide may delay the appearance of resistance to isoniazid when the drugs are used in combination (6). Only 2 of the 4 patients who received this form of therapy had "positive" sputum long enough to determine effects on isoniazid susceptibility. In one patient, the tubercle bacilli remained completely susceptible to isoniazid; and in the other patient there were but five colonies on the medium con-

TABLE 2

PATIENTS RE-TREATED WITH PYRAZINAMIDE IN ASSOCIATION WITH OTHER THERAPY

PATIENT	STREPTOMYCIN SUSCEPTIBILITY OF ORGANISMS	CONCOMITANT THERAPY	CLINICAL CHANGE	SPUTUM "CONVERSION"	ROENTGENOGRAPHIC CHANGE	LENGTH OF TREATMENT (WEEKS)
1. H.L.	R	Streptomycin daily	7*	No	4	9
2. W.B.	S	Streptomycin daily	2	Yes	2	6
3. J.W.H.	R	Streptomycin twice weekly	4	No	4	12
4. V.M.	S	Streptomycin twice weekly	1	On microscopic examination only	6	12
5. T.G.	S	Streptomycin twice weekly	7†	No	4	6
6. R.D.C.	S	Streptomycin twice weekly	2	Yes	3	13
7. W.K.B.	S	Streptomycin twice weekly	4	No	4	6
8. A.B.	R	Dihydrostreptomycin twice weekly	4	No	5	15
9. J.M.	S	Dihydrostreptomycin twice weekly	2	Yes	2	11
10. H.S.	S	Dihydrostreptomycin twice weekly	4	No	5	10
11. C.W.	R	PAS daily	4	No	5	8
12. B.K.	R	Streptomycin twice weekly plus PAS daily	4	No	4	17
13. S.M.	R	Streptomycin twice weekly plus PAS daily	2	No	4	19
14. C.D.	S	Streptomycin twice weekly plus PAS daily	3	On microscopic examination only	3	17
15. H.R.	S	Isoniazid, 300 mg. daily	2	No	2	16
16. C.C.	S	Isoniazid, 300 mg. daily	3	No	3	16
17. H.W.	S	Isoniazid, 300 mg. daily	3	Yes	3	16
18. M.R.	R	Isoniazid, 300 mg. daily	3	Yes	3	16

* Died of complications of paraplegia.

† Died of lymphosarcoma of stomach.

4 = no change; 3, 2, 1, = slight, moderate, and marked improvement; 5, 6, 7 = slight, moderate, and marked worsening.

R = resistant

S = susceptible

taining 5 γ of isoniazid per ml., compared with a 3-plus growth in the control medium, after four months of therapy.

Hepatitis: The one serious toxic reaction probably due to the therapy was in the patient who received pyrazinamide alone and who developed jaundice with evidence of parenchymatous liver damage. Five other patients of 20 patients in whom routine tests were done had evidence of mild hepatic dysfunction as manifested by slightly elevated bromsulfalein retention, with one also showing a prothrombin time of 28 per cent of the normal value. In the latter patient, therapy was discontinued because of toxicity. The prothrombin time quickly returned to normal while the bromsulfalein tests returned to normal more slowly. One seriously ill patient complained of abdominal cramps following the medication, but he had had similar, although less severe, complaints following previous other oral therapy.

GENERAL COMMENT

The present results with pyrazinamide do not seem quite as favorable as those reported by the original investigators. In the patients of the present study who received pyrazinamide alone, the results were somewhat better in the original treatment group, clinically and roentgenographically, than in the re-treatment group but not good enough to advise the use of this drug alone except, perhaps, in special circumstances. In the patients who received concurrent therapy with other antimicrobial agents, only those with streptomycin-susceptible bacilli showed improvement roentgenographically and by sputum "conversion," with the exception of the one patient with streptomycin-resistant organisms who received concurrent isoniazid. Such patients probably would have been expected to improve had pyrazinamide not been added. There was an insufficient number of patients with streptomycin-susceptible organisms whose sputum remained positive for tubercle bacilli on completion of therapy to determine the effects of pyrazinamide on delaying resistance to streptomycin. No conclusion can be drawn from the few patients who received concurrent isoniazid and pyrazinamide whether pyrazinamide delays the appearance of resistance to isoniazid.

SUMMARY

Pyrazinamide appeared to be of benefit to some patients with active pulmonary tuberculosis. It was more effective in favorable original treatment cases than in failures of other antimicrobial therapy. When given concurrently, with streptomycin and/or para-aminosalicylic acid, it had some favorable effect on patients with streptomycin-susceptible organisms, but not on those with streptomycin-resistant organisms. Pyrazinamide and isoniazid may be worth trying in the latter type of infection. The toxicity of pyrazinamide did not appear to be unduly significant. One patient of the 29 developed hepatitis; 5 of 20 others developed slightly abnormal liver function tests. The patient with hepatitis and one of the patients with abnormal hepatic function improved promptly on cessation of the drug administration; otherwise, the medication was continued without ill effect. *In vitro* susceptibility tests were of no aid in the therapeutic use of pyrazinamide.

SUMARIO

Observaciones acerca de la Piracinamida (Aldinamida) en la Tuberculosis Pulmonar

La piracinamida pareció ser de beneficio para algunos enfermos que tenían tuberculosis pulmonar activa. Fué más eficaz en los casos que habían respondido favorablemente al tratamiento primitivo que en aquéllos en que había fracasado otra terapéutica antimicrobiana. Cuando se administró simultáneamente con la estreptomicina y/o el ácido para-

aminosalicílico, ejerció algún efecto favorable en los enfermos que tenían microbios estreptomicinosusceptibles, pero no en los que tenían gérmenes estreptomicinorresistentes. Valdría la pena probar la piracinamida y la isoniacida en esta última clase de infección. La toxicidad de la piracinamida no pareció ser demasiado exagerada. Uno de los 29 enfermos manifestó hepatitis; 5 de otros 20 acusaron pruebas ligeramente anormales de la función hepática. El enfermo de hepatitis y uno de los que tuvieron función hepática anormal mejoraron prontamente al cesar la administración de la droga; aparte de eso, se continuó la medicación sin efectos adversos. Las pruebas *in vitro* de la susceptibilidad no fueron de la menor ayuda en el uso terapéutico de la piracinamida.

RESUME

Observations sur la pyrazinamide (Aldinamide) dans la tuberculose pulmonaire

La pyrazinamide a produit un effet favorable chez certains malades atteints de tuberculose pulmonaire en activité. Son efficacité était plus marquée quand un traitement initial avait été favorable que dans les cas où une autre thérapie antibactérienne avait échoué. Quand elle était administrée concurremment avec la streptomycine et l'acide para-aminosalicylique, ensemble ou séparément, elle faisait preuve d'une certaine efficacité chez les patients porteurs de bacilles streptomycino-sensibles, mais elle était sans action en présence de bacilles streptomycino-résistants. La pyrazinamide et l'isoniazide méritent d'être essayées dans ce dernier type d'infection. La toxicité de la pyrazinamide n'a pas paru d'une importance excessive. Parmi 29 malades, 1 a présenté de l'hépatite; dans 20 autres cas, les tests de la fonction hépatique ont été trouvés légèrement anormaux dans 5 cas. Le malade atteint d'hépatite et un des patients dont la fonction hépatique était anormale ont présenté une amélioration rapide quand on a cessé l'administration du médicament; d'ailleurs, la médication a été continuée ensuite sans produire d'effets défavorables. *In vitro*, les tests de sensibilité n'ont été d'aucune utilité quant à l'usage thérapeutique de la pyrazinamide.

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FATAL ALLERGIC REACTION TO PARA-AMINOSALICYLIC ACID[1]

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Toxic reactions to para-aminosalicylic acid (PAS) have been differentiated from those due to hypersensitivity to the drug by Warring and Howlett (1), also by Hayes and Weiss (2). According to the latter authors, the incidence of the allergic response may be as high as 4 per cent among patients treated with PAS. The following case is presented to emphasize the serious form which allergic reactions to PAS may assume.

CASE REPORT

On December 23, 1952, a white male, 47 years of age, was admitted to Wm. H. Maybury Sanatorium with a four-month history of weakness and ease of fatigue. Admission chest roentgenograms revealed a far advanced tuberculous process with dense infiltrations of the mixed type throughout the upper half of both lung fields and bilateral cavitation. He was moderately underweight but otherwise in good condition. The admission blood pressure was 130 systolic and 90 diastolic in mm. of mercury. Admission temperature was 100.0°F.; pulse, 104; and respirations, 24 per minute. The sputum was positive for acid-fast bacilli on microscopic examination. The patient showed a positive cutaneous reaction to the 1:10,000 dilution of tuberculin. Admission blood examination was reported as follows: hemoglobin, 16.0 gm.; total leukocytes, 12,100 per cu. mm., with 80 per cent neutrophils, 14 per cent lymphocytes, and 6 per cent monocytes. Urine examination was normal; serologic test for syphilis (Kahn) was negative.

On December 25, the patient was started on streptomycin, 1 gm. three times weekly, and sodium para-aminosalicylate, 16.5 gm. daily in three divided doses. Isoniazid, 100 mg. twice daily, was added on December 29 (figure 1). Therapy with all three drugs was continued without incident until January 15, 1953, when the patient complained of soreness and throbbing at the site of the streptomycin injection. On January 17, his temperature rose to 102°F., and streptomycin was discontinued. On January 18, he received his usual dose of isoniazid and 5.5 gm. of sodium PAS. On the nineteenth and twentieth he received isoniazid alone. On the latter date his temperature returned to normal. On January 21 at 9:00 a.m., he received isoniazid plus a test dose of 5.5 gm. of sodium PAS. This was followed in approximately two hours by a generalized erythematous macular rash, nausea, and vomiting. His temperature rose to 103.8°F. PAS and isoniazid were discontinued. A differential blood count revealed 10,500 leukocytes per cu. mm., with 59 per cent neutrophils, 27 per cent eosinophils, 2 per cent basophils, and 12 per cent lymphocytes. The acute clinical reaction responded moderately to Benadryl, 50 mg. four times daily, and to daily starch baths, with some reduction of the swelling of the skin and the generalized pruritus. The patient's temperature declined to 99.4°F. on January 22 and remained at about that level for eight days. On January 27, slight edema of the face was first noted, and on January 28 the patient complained of diarrhea and later in the day was nauseated. The temperature rose to 101.4°F. on January 30, then gradually declined, eventually reaching a subnormal level (figure 1).

The patient seemed to show general improvement until February 5, 1953, when he complained of dryness of the throat and some increase in his general feeling of malaise. The following day he had marked thirst and drank approximately six quarts of water. That evening he was confused, wandering about his room, and had an involuntary liquid defecation.

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[1] From the Wm. H. Maybury Sanatorium (Detroit Municipal Tuberculosis Sanatorium), Northville, Michigan.

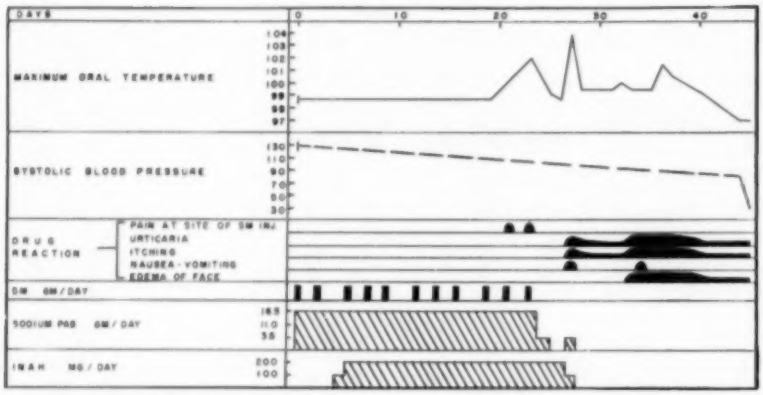

FIG. 1. Fatal allergic reaction to PAS. Clinical course.

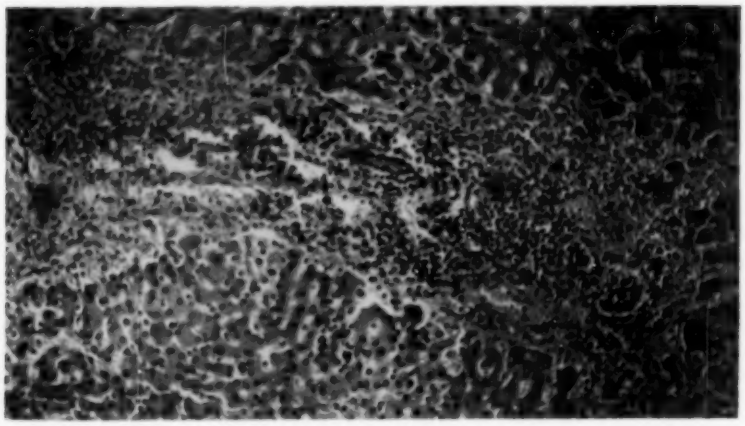

FIG. 2. Section of liver showing slight periportal fibrosis and infiltration of plasma cells, polymorphonuclear neutrophils and eosinophils. (× 150)

When seen by his physician (M. D. K.) at 8:30 a.m. on February 7, the patient was unconscious and could not be roused.

The blood count at that time was reported as follows: hemoglobin, 14.75 gm.; erythrocytes, 3,580,000; total leukocyte count, 21,500, with 63 per cent neutrophils, 6 per cent eosinophils, 1 per cent basophils, 29 per cent lymphocytes (1 plasma cell), and 1 per cent monocytes. Many neutrophils showed toxic granulation.

Intravenous glucose in saline was given along with 20 ml. of adrenal cortex extract (Eschatin). At 1:00 p.m., the patient's blood pressure was 80 systolic and 50 diastolic in mm. of mercury. It gradually fell to a final reading of 30 systolic and 20 diastolic at 8:00 a.m. on February 8. In addition to intravenous Eschatin, the patient received 10 ml. of Eschatin subcutaneously, with repeat doses of 2 ml. at three-hour intervals until his death. Also the patient was given 1 ml. of aqueous epinephrine plus 1 ml. of epinephrine in oil every three hours intramuscularly. At 4:30 a.m. on February 8, he voided for the first time in

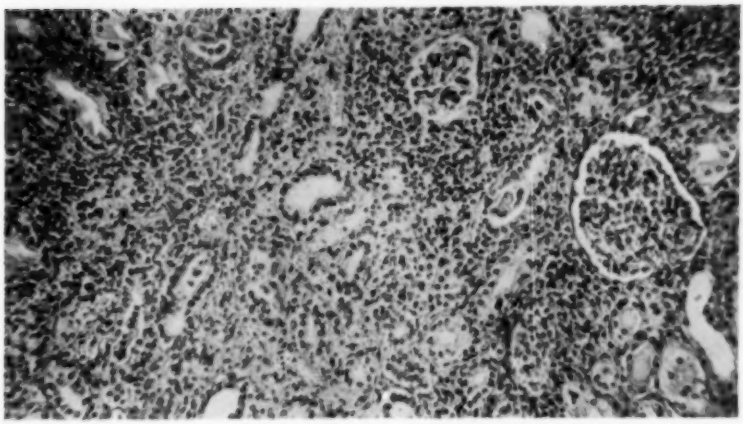

FIG. 3. Section of kidney showing area of recent scarring with infiltration of plasma cells, polymorphonuclear neutrophils and eosinophils. (× 150)

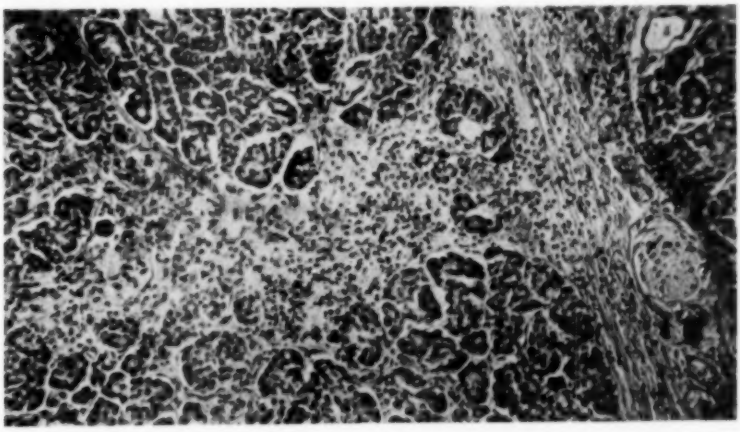

FIG. 4. Section of pancreas showing area of recent scarring with infiltration of plasma cells, polymorphonuclear neutrophils and eosinophils. (× 150)

twenty-four hours. His temperature was subnormal the last two days. Respirations ceased at 8:40 a.m. on February 8, 1953. The clinical impression of cause of death was acute adrenal failure and exfoliative dermatitis, following hypersensitization to PAS.

Post-Mortem Examination

Macroscopic examination: The skin over the entire body varied from yellow-red to light purple in color. In several places the superficial epithelium had exfoliated. The heart weighed 270 gm. and showed dilatation of both ventricles. The musculature was flabby. Several small caseous lesions were found in the upper lobes of both lungs, with a 2 cm. cavity in the right upper. Both lungs appeared congested and edematous. Foul-smelling

yellow fluid was found in the fundus of the stomach. No ulceration was found in the gastrointestinal tract. The pancreas was firm, pink in color. The liver weighed 1,700 gm. and was yellow-brown, mottled with occasional red areas; the organ was flabby, with indistinct lobulations. Both kidneys were enlarged; the left weighed 200 gm., the right 180 gm. Exteriorly and on section they presented a mottled yellow-red appearance. The cortex was wide, measuring 8 mm. in thickness. There were no other gross findings of particular interest.

Microscopic examination: There was extensive fragmentation of the myocardial fibers. Some of the lung sections showed caseous lesions. In others the alveoli were filled with serous fluid. Fibrino-purulent exudate was found in the alveoli about some of the bronchioles. The gastric mucosa was infiltrated with plasma cells and polymorphonuclear leukocytes, many of which were eosinophils. The liver sections showed slight periportal fibrosis with an extensive infiltration of plasma cells and polymorphonuclear leukocytes in the periportal areas (figure 2). Many of the latter cells were eosinophils. Sections of the kidneys showed numerous areas of recent scarring throughout the cortex (figure 3). These areas were infiltrated with plasma cells and polymorphonuclear leukocytes, most of the latter being eosinophils. The pancreas likewise showed numerous areas of recent scarring which were infiltrated with plasma cells and polymorphonuclear leukocytes, mostly eosinophils (figure 4). There were no recognizable Langerhans' islands (5 sections of pancreas were examined). A few small infiltrations of inflammatory cells were found in the medulla of each adrenal gland.

COMMENT

It appears that the patient died in shock which came on as a delayed result of a 5.5 gm. dose of sodium para-aminosalicylate (PAS) in the presence of hypersensitivity to this drug. The clinical picture of lassitude, nausea, vomiting, diarrhea, collapse, coma, and death resembled that of acute adrenal cortical insufficiency. The patient's severe thirst, noted two days before death, as well as the complete absence of Langerhans' islands in the pancreas suggest the possible acute onset of diabetes. The present case serves to emphasize the need for caution in administering test doses of antimicrobials, particularly PAS, to patients suspected of being sensitized to one or another of these drugs.

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NEAR-FATAL SHOCK FROM PAS FOLLOWED BY GUILLAIN-BARRÉS SYNDROME[1]

K. G. BULLEY

(Received for publication August 21, 1953)

The following report is concerned with an instance of peripheral vascular collapse of serious degree which occurred following the administration of para-aminosalicylic acid (PAS). The incident was followed by the development of the Guillain-Barrés syndrome with eventual recovery.

CASE REPORT

On August 14, 1951, a white 29-year-old housewife was readmitted to Kane County Springbrook Sanitarium for intrapleural pneumonolysis of an adhesion, at the base of which a new soft tuberculous infiltrate had recently appeared. No chemotherapy had been used in her previous treatment, which dated back to 1943. She was placed on sodium para-aminosalicylic acid (PAS), 4 gm. three times daily by mouth, and dihydrostreptomycin, 1 gm. intramuscularly twice a week. She took the drugs without evidence of allergy for ten days.

An uneventful intrapleural pneumonolysis was done on August 23, 1951. Following surgery she did not resume taking PAS until August 26, 27, and 28. On each of these days she took 4 gm. of PAS at breakfast only. On each occasion this was followed by severe headache, nausea, chilling, and malaise, with temperature elevations to 102.4°F. No other cause for her symptoms could be ascertained, and PAS was discontinued. Some packettes of PAS remained in her bedside cabinet and, on September 4, on her own initiative she took 4 gm. of sodium PAS with breakfast. Within ten minutes she developed severe headache, nausea, vomiting, cramping pains in the abdomen and legs, and diarrhea. This was followed by severe pain in her back, chilling, blurred vision, and conjunctival injection.

In view of the course of her previous reactions, it was believed that her symptoms would subside later in the day, and she was given codeine, 0.03 gm. hypodermically at 10 a.m., 1 p.m., and 4 p.m. She became worse, with severe pain in her back, headache, and general aching; at 5:30 p.m., she was given Demerol, 50 mg. hypodermically, and Pyribenzamine, 50 mg. orally. At 6 p.m. she had a large brownish emesis. She moaned continuously, and the skin over her entire body showed a red flush similar to scarlatina. The abdominal cramps and the pains in her legs and back became more severe. Her pulse was rapid but of good quality. At 9:30 p.m., Demerol, 50 mg. hypodermically, was repeated. At this time her temperature was 101°F. and the pulse was 96 per minute. Morphine, 0.005 gm. hypodermically, was given for pain at 10:45 p.m.

At 11 p.m., the physician was called back to see her. There was no perceptible radial pulse or blood pressure. She moaned continuously, and her whole body had a deep flush. She was in extreme shock, and her condition was critical. During the following thirty-six hours, continuous intravenous medication was required to keep the patient alive and the systolic blood pressure near 60 mm. of mercury, with her pulse approximately 120 and temperature 102.6° to 103.6°F. She received 6 units of plasma, 1,000 cc. of 5 per cent glucose in saline, 1,000 cc. of 10 per cent glucose in water, and 1,000 cc. of 5 per cent glucose in water.

During this thirty-six-hour period, a total of only 170 cc. of urine was obtained on four catheterizations. The specimens showed a high specific gravity (up to 1,032); 2- and 3-plus albumin; and a few erythrocytes, pus cells, and granular casts per high-power field.

In this thirty-six-hour period, the patient received the following medications: epinephrine hydrochloride (1:1,000), 0.18 ml. (4 doses hypodermically); morphine, 0.01 gm. (5 doses

[1] From Kane County Springbrook Sanitarium, Aurora, Illinois.

intramuscularly); Benadryl, 10 mg. (4 doses intravenously); Benadryl, 50 mg. (2 doses orally); corticotropin, 25 mg. (4 doses hypodermically); Cortate, 5 mg. (one dose hypodermically); and oxygen by nasal catheter.

By 8 a.m. of September 6, 1951, the intravenous drip could be discontinued and the blood pressure held at 74 systolic and 40 diastolic, gradually rising during the day to 106 systolic and 60 diastolic. The corticotropin, morphine, and Benadryl were continued and the patient improved. At 4 p.m. on September 6, 400 cc. of urine were obtained by catheter. This showed a specific gravity of 1,010; albumin, 2 plus; sugar trace; diacetic 0; and acetone 0. Microscopic examination showed 4 or 5 erythrocytes, 5 or 6 pus cells, and 4 granular casts per high-power field. Diminishing amounts of albumin were present in the urine for one month. Erythrocytes disappeared in a few days.

On the afternoon of September 6, the patient developed a complete bilateral facial palsy, marked weakness of the right arm and shoulder, and inability to move either foot. Her back was slightly stiff, and there was abdominal distension. Her general condition showed marked improvement.

On September 10, the cerebrospinal fluid showed a cell count of 154; 97 per cent lymphocytes; albumin, 3 plus; total protein, 119 mg. per 100 ml. On September 19, the cerebrospinal fluid showed a cell count of 5; protein, 72 mg. per 100 ml.; albumin, 2 plus.

On September 18, the consultant on neurology, Dr. Alan Lieberman, gave the following report:

> "Neurological examination discloses widespread involvement of neuraxis, motion paresis of both legs, right arm, and, less so, left arm. There are no neck signs. Fundus negative. Has complete bilateral paralysis of facial nerves. No other cranial nerves involved. All reflex (deep tendon) physiology is absent except for those of left arm—abdominals absent. Does not have neurogenic bladder. Bilateral peroneal foot drop. Has definite hypo-algesis and hypo-esthaesia of both feet to above the ankle—posterior column markedly involved. Absent vibration, position, and deep sensibility of legs. Has neuronitis and plexitis, and radiculitis. Impression: Guillain-Barrés neuromyelo-radiculo-neuritis. Prognosis: Excellent for recovery."

The patient lost all of her hair. This eventually grew out again. There was marked scaling and itching of her skin approximately one week after the onset of her symptoms. Severe pain was present in her feet and hands for many weeks. The complete bilateral facial paralysis remained for six weeks, and function gradually returned to normal at twelve weeks. She was unable to walk for more than seven months.

With intensive vitamin therapy and physiotherapy during a period of eighteen months, the patient made a gradual and almost complete recovery of both muscle and nerve function. A flexion contracture of the fifth right finger will require surgical correction.

COMMENT

This case report is presented to exhibit the serious aspects of allergic reactions to PAS and to emphasize the need for immediate measures to combat shock as soon as severe symptoms appear. Mild early reactions to PAS give no reliable indication of the possible severity of subsequent reactions. Intensive anti-shock therapy applied sooner would presumably have reduced the extent of the neurologic damage in this case and might have averted the profound shock.

SUMMARY

A case is reported of the development of severe peripheral vascular collapse as a manifestation of hypersensitivity to para-aminosalicylic acid (PAS). The prolonged shock was followed by the appearance of the Guillain-Barrés syndrome, which eventually disappeared.

SUMARIO

Colapso Vascular como Manifestación de Hipersensibilidad al Acido Para-Aminosalicílico

Comunícase un caso de producción de grave colapso vascular periférico como manifestación de hipersensibilidad al ácido para-aminosalicílico (PAS). El choque prolongado fué seguido de la aparición del síndrome de Guillain-Barré, que desapareció con el tiempo.

RESUME

Shock presque fatal dû PAS suivi par le syndrome de Guillain-Barrés

Un cas de collapsus vasculaire périphérique sévère par hypersensibilité à l'acide para-aminosalicylique (PAS) a été observé. A l'état de choc prolongé a succédé un syndrome de Guillain-Barrés, qui a disparu par la suite.

THE GOITROGENIC EFFECT OF PARA-AMINOSALICYLIC ACID DURING THE THERAPY OF PULMONARY TUBERCULOSIS[1,2]

Report of Eleven Cases

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INTRODUCTION

Several reports have now appeared describing thyroid enlargement occurring during therapy with para-aminosalicylic acid (1–4). This effect is evidently related to the ability of para-aminosalicylic acid (PAS) to depress iodine uptake by the thyroid gland. Experimental work has shown that, *in vitro*, PAS is capable of binding a large amount of iodine from an iodine solution (5). In rats, cellular hyperplasia of the thyroid gland has been demonstrated after feeding with PAS and aminophenol (7). Hanngren (6), working with human subjects, has demonstrated cessation of radioactive iodine uptake by the thyroid gland forty-five minutes after starting an intravenous transfusion of PAS. This inhibitory effect lasted for fifteen hours, after which the gland was again able to take up iodine. Similar findings have been reported by Sutherland (8) in this country. It is of interest that Promizole, another antituberculous drug, has also produced thyroid enlargement both in experimental animals (9) and in humans (10).

Clinical Observations

During the two- and one-half-year period from July, 1950 to January, 1953, 11 cases of goiter have developed at Trudeau Sanatorium among patients who received PAS. No goiters were observed among patients who received no PAS, or among the employees and staff of the Sanatorium.

During the period under observation, prolonged streptomycin-PAS therapy was the standard chemotherapy given to the majority of patients, although in 1952 isoniazid was used to some extent both alone and in combination with streptomycin or PAS. However, PAS was the only regular medication (with the exception of multiple vitamin preparations) given to all those who developed goiter. A summary of the chemotherapy regimens among these 11 patients follows:

Streptomycin plus PAS	9
Isoniazid plus PAS	1
Streptomycin plus isoniazid plus PAS	1
Total	11

Deliberate interruption of drugs to demonstrate a cause and effect relationship was considered unjustified after it was recognized that this might impair therapeutic effectiveness. However, in 3 patients whose therapy was either interrupted or terminated, disappearance of the goiter was observed within two to three weeks.

Study of the 11 persons who developed goiter provided the data presented in table 1.

Nine of the 11 goiters occurred in females, whereas the patient population in the Sanatorium as a whole was approximately equally divided between men and women.

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[1] From Trudeau Sanatorium, Trudeau, New York.

[2] This investigation was supported by a fellowship grant to the senior author from the Lawrason Brown Memorial Fund.

TABLE 1

SUMMARY OF THE DATA IN 11 PATIENTS WHO DEVELOPED GOITER DURING PAS THERAPY

PATIENT	AGE	SEX	CHEMOTHERAPY	DURATION BEFORE GOITER	CLINICAL MYXEDEMA	SERUM CHOLESTEROL	BASAL METABOLISM RATE	ANTI-GOITER THERAPY	RESULTS
						mg. per 100 cc.			
J. H.	24	F	Streptomycin-PAS	9 months	No	—	—	None	Goiter remains stationary
H. Z.	50	F	Streptomycin-PAS	8 months	No	237	−11	None	Goiter remains stationary
M. T.	34	F	Streptomycin-PAS	6 months	No	—	—	None	Goiter remains stationary
M. J.	22	M	Streptomycin-PAS	7 weeks	No	222	−6	Streptomycin-PAS stopped	Goiter disappeared in 3 weeks
P. O.	22	F	Streptomycin-PAS	9 months	No	—	—	Streptomycin-PAS stopped	Goiter disappeared in 2 weeks
R. L.	45	F	Streptomycin-PAS	5 months	No	170	−1	None	Goiter disappeared spontaneously but returned 6 weeks later
V. S.	36	F	Streptomycin-PAS	4 months	No	230	−20	None	Goiter disappeared spontaneously but returned 4 weeks later
P. G.	26	F	Streptomycin-PAS	12 months	Yes	326	—	Iodine Thyroid	No effect Abolished clinical myxedema in in 5 weeks
R. A.	20	F	Isoniazid-PAS	5 months	No	172	−12	Iodine Thyroid	No effect Goiter disappeared within 1 month
R. R.	20	M	Streptomycin-PAS-Isoniazid	9 months	No	228	—	Thyroid	Goiter smaller 2 weeks after start of thyroid
A. B.	40	F	Streptomycin-PAS	5 months	Yes	305	−12	Thyroid	Improvement apparent within 2 weeks of starting thyroid

No specific family history of goiter could be elicited among this group, nor was any one geographic area represented among them, other than the fact that most came from states in the northeastern portion of the United States, as did the majority of patients at the Sanatorium.

Nothing unusual could be found regarding the form of PAS which these patients received. All had received the standard regimen of 4 gm. of PAS three times daily, and the three forms of the drug then in use at the Sanatorium were all represented, namely, sodium PAS salt in solution, tablet form, and powder.

The onset of thyroid swelling varied from four to twelve months (average 6.7 months) after the start of PAS therapy, with the exception of one patient (M. J.) whose goiter appeared in seven weeks. In spite of this delay in the appearance of goiter in the majority, prolongation of therapy appeared to be of little importance in itself as an etiologic factor. Combined streptomycin-PAS therapy was administered to a total of 72 patients for six to twelve months and to 190 patients for twelve months or more during the period under review.

Clinical and laboratory observations provided evidence of reduced thyroid function, as might be expected. Cholesterol determination in 8 of these patients showed abnormally high values in 2, and results in the upper range of normal in an additional 4 patients. Two of these patients (P. G. and A. B.) developed clinical myxedema which showed no response to iodine medication but prompt benefit from thyroid extract.

Two cases illustrating points of particular interest are detailed below.

Case Reports

Case 1: P. G. was a 26-year-old female teacher with moderately advanced pulmonary tuberculosis for which she received streptomycin and PAS (12 gm. daily) beginning in October, 1951. She underwent a wedge resection for persisting cavity in the left upper lobe in July, 1952, at which time her streptomycin was increased from 1 gm. twice weekly to 1 gm. daily. She tolerated the drugs well and made satisfactory progress, but on October 17, 1952, after twelve months of chemotherapy, a goiter was noticed; her neck circumference was 13.5 inches (figure 1). She was given Lugol's solution, 2 cc. per day (equivalent to 0.26 gm. of iodine) and the streptomycin and PAS were continued. Although the Lugol's dosage was later increased to 3 cc. per day, the goiter persisted. Two months after the goiter was first observed, her neck measurement had increased by one inch, to 14.5 inches. She had a typical myxedematous facies and had gained 10 pounds in weight. Serum cholesterol was 326 mg. per 100 cc. On December 27, 1952, medication with thyroid, 0.03 gm. daily, was started and two weeks later this was increased to 0.065 gm. daily. Five weeks after the start of thyroid therapy her facial appearance was back to normal, her neck measured 13.5 inches, and she had lost 9 pounds in weight. Serum cholesterol now measured 240 mg. per 100 cc. Streptomycin and PAS therapy was not interrupted at any time but was continued along with the desiccated thyroid. Although she became symptom free, the gland did not return to normal size during streptomycin-PAS therapy.

This case demonstrates clearly the ineffectiveness of iodine in preventing the development of hypothyroidism in these patients, whereas thyroid in moderate doses will relieve the condition.

In only one other case has myxedema been demonstrated. This was a woman (A. B.) of forty who after five months of daily streptomycin and PAS (12 gm. daily) developed a goiter. Chemotherapy was continued, but within a month she had the typical myxedematous facies, felt lethargic, and spent most of the day sleeping. Her serum cholesterol was 305 mg. per 100 cc. The basal metabolism rate was −4. She also showed a rapid response to thyroid medication.

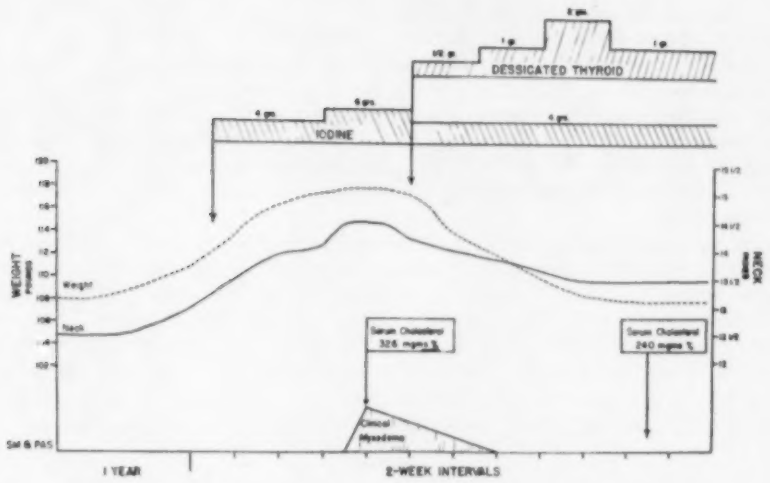

FIG. 1. Schematic representation of the clinical course in patient P.G.

Case 2. R. S. was a 45-year-old female secretary with moderately advanced pulmonary tuberculosis. She started streptomycin (1 gm. twice weekly) and PAS (12 gm. daily) in May, 1952. Five months later, in October, a goiter was noticed for the first time. Her neck measured 13.1 inches. Streptomycin and PAS were continued, and no therapy was given for the goiter. The latter remained unchanged for six weeks, and in mid-December it disappeared. Her neck then measured 12 inches. In early February, the goiter reappeared and her neck size remained at 13 inches. In this patient also, streptomycin and PAS have been continued without interruption.

This case and one other (F. S.) are the only 2 in which the goiter underwent spontaneous remission, which lasted, respectively, six weeks and eight weeks before the goiter reappeared.

Observations in this group of patients have not confirmed Librach's statement (3) that the longer PAS administration is continued after the appearance of thyroid swelling, the greater is the risk of myxedematous changes. The patient (R.S.), whose case was presented above, was observed for five months after development of goiter, and, despite the continuation of PAS without any antithyroid medication, her condition did not progress into myxedema. Three other patients observed for two months after development of goiter showed no signs of hypothyroidism in spite of continuation of streptomycin-PAS treatment. In the 2 cases in which myxedema occurred, it appeared within a month of the goiter development.

COMMENT

Although the development of clinical goiter among patients receiving PAS might be anticipated in view of its demonstrated ability to block iodine uptake by the thyroid, surprisingly few such cases have been reported in the literature. In the present group of 11 patients, a relationship of PAS administration to the development of goiter is indicated by three observations: first, the fact that PAS was the only medication regularly taken

by all; second, the absence of goiter among patients, employees, and staff, who did not receive PAS; and, finally, the disappearance of goiter when PAS was stopped.

From a therapeutic standpoint, administration of iodine produced no effect in the 2 persons in whom it was tried, thus differing from a simple iodine-deficient goiter. Small doses of thyroid extract, however, gave prompt improvement in the 4 patients to whom it was given, with disappearance of clinical signs of myxedema and regression of the size of the gland. It is noteworthy, however, that continuation of PAS after the appearance of goiter did not lead to clinical myxedema or to further enlargement of the gland in several of these patients.

Although the group with goiter represented only a small segment of the total number of patients receiving prolonged PAS therapy, no specific predisposing factors could be elicited among these persons. A possible relationship of goiter to amino acid metabolism is suggested by the demonstration that PAS in the diet neutralizes ascorbic acid (vitamin C) (12), which is necessary for the synthesis of diiodotyrosine (13) and consequently of thyroxine. Since mild vitamin C deficiency is relatively common in patients with active pulmonary tuberculosis (11), investigation of its relationship to PAS therapy and to thyroxine synthesis might clarify the factors in production of this type of goiter.

SUMMARY

Eleven cases of thyroid enlargement occurring during para-aminosalicylic acid (PAS) therapy are reported, with the development of myxedema in 2 patients. Iodine therapy was ineffective in relieving the condition, whereas thyroid in moderate doses was curative in the 4 cases in which it was given. There appears to be no contraindication to continuing PAS therapy along with thyroid when necessary.

SUMARIO

El Efecto Bocígeno del Acido Para-Aminosalicílico Durante la Terapéutica de la Tuberculosis Pulmonar

Comunícanse 11 casos de hipertrofia tiroidea observados durante el tratamiento con ácido para-aminosalicílico (PAS), con aparición de mixedema en 2 enfermos. La yodoterapia resultó ineficaz para aliviar el estado, mientras que el tiroides a dosis moderadas se mostró curativo en los 4 casos en que se administrara. No parece que haya contraindicación a la continuación del tratamiento con PAS junto con tiroides cuando es necesario.

RESUME

Action goitrogène de l'acide para-aminosalicylique au cours de la thérapie de la tuberculose pulmonaire

Onze cas d'hypertrophie de la thyroïde, qui se sont manifestés au cours d'un traitement par l'acide para-aminosalicylique (PAS), sont rapportés. Le myxoedème s'est développé chez 2 malades. La thérapie iodée s'est montrée inefficace, tandis que la thyroïde à doses modérées a procuré la guérison dans les 4 cas où elle a été pratiquée. Il n'apparaît pas qu'il y ait de contre-indication à poursuivre le traitement par le PAS, en association avec l'administration de thyroïde si nécessaire.

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Notes

STUDY OF THE VIRULENCE OF ISONIAZID-RESISTANT TUBERCLE BACILLI IN GUINEA PIGS AND MICE

A Preliminary Report

Contradictory reports from several reputable investigative laboratories regarding the question of animal virulence of isoniazid-resistant tubercle bacilli have indicated that this subject is one of increasing diagnostic interest and importance.

Karlson and Ikemi[1] and Goulding and associates[2] published data which clearly demonstrated that isoniazid-resistant tubercle bacilli lost no virulence for mice. On the other hand, Grunberg[3] stated that isoniazid-resistant tubercle bacilli did not cause progressive disease in guinea pigs or mice. Recently, Peizer and her group[4] reported that isoniazid-resistant tubercle bacilli retained pathogenicity for the guinea pig.

The present report presents the results of two independently conducted experiments which may shed some light on the controversial subject of the animal virulence of isoniazid-resistant variants. The first experiment was conducted in the fall of 1952, at the Army Medical Service Graduate School, Walter Reed Army Medical Center, Washington, D. C.; the second was conducted early last winter in the Research and Development Branch, Fitzsimons Army Hospital, Denver, Colorado.

In the experiment conducted at the Army Medical Service Graduate School, acid-fast, isoniazid-susceptible bacilli culturally identified as *M. tuberculosis*, and determined to be pathogenic for the guinea pig, were transferred from Löwenstein medium,[5] free of isoniazid, to Tween[6]-albumin medium,[7] also without isoniazid, and grown to a suitable density. Transfers were made from the Tween medium to Löwenstein medium containing known concentrations of isoniazid and, after suitable growth of the selected isoniazid-resistant variants had developed, transfers were again made to Tween-albumin medium without isoniazid. Each resistant strain was grown to a density equal to 5 mg. of bacilli by wet weight in 1.0 ml. of the culture medium. The isoniazid-resistant strains were inoculated subcutaneously in the groin of each of 3 animals, each animal receiving 5.0 mg. of bacilli by wet weight. One pig from each series was sacrificed after three weeks, one after six weeks, and one after nine weeks. Cultures on Löwenstein medium were made from tissue homogenates of the spleen, liver, and lungs.

In the second experiment, conducted in the Research and Development Branch, Fitzsimons Army Hospital, acid-fast strains culturally consistent with *M. tuberculosis* and reported by the laboratory as being resistant to 100 γ of isoniazid per ml. were transferred to modified Petragnani egg medium.[8] After suitable growth, a transfer was made to Tween-albumin medium containing no isoniazid, and, after a reasonable density had

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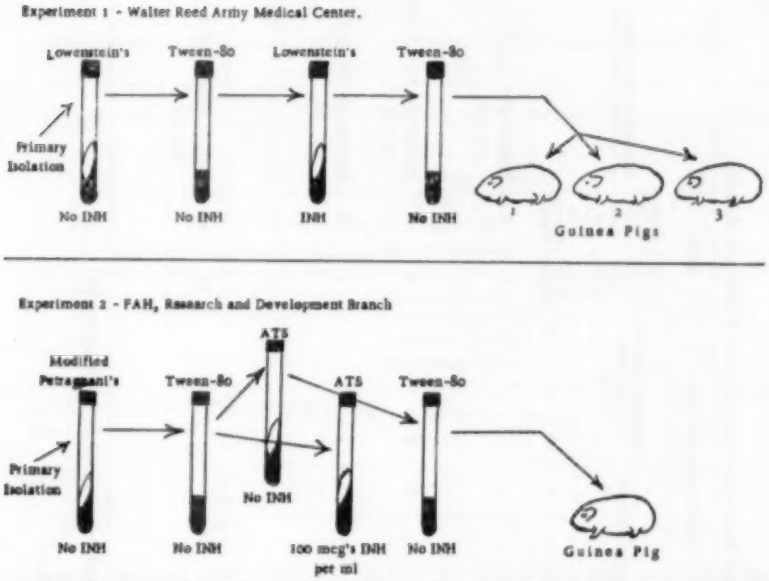

FIG. 1. Experimental technique used in studying the virulence in guinea pigs of isoniazid-resistant tubercle bacilli.

developed, transfers were made to one tube of American Trudeau Society (ATS) medium[9] without isoniazid and to another tube containing isoniazid, 100 γ per ml. Subculture from ATS medium containing no isoniazid was made to Tween-albumin medium containing no isoniazid and, after suitable density had developed, each strain was inoculated subcutaneously into the groin of a guinea pig.

It is important to compare again the experimental techniques of the two experiments (figure 1). In the experiment performed at Walter Reed, the subcultures passed from a medium containing isoniazid to a medium containing no isoniazid, and subsequently were inoculated into the animals. In the second experiment, conducted at Fitzsimons Research and Development Branch, the isoniazid-containing medium was by-passed, and the bacilli introduced to the animals were not subjected to isoniazid during an experimental procedure.

The results of the Walter Reed experiment (table 1) indicate that none of the animals inoculated with the eleven strains selected as resistant to 50 γ of isoniazid per ml. showed evidences of visceral disease, whether sacrificed at three weeks, six weeks, or at nine weeks. A single strain selected as resistant to 25 γ of isoniazid per ml., but susceptible to 50 γ, also failed to produce progressive disease in any of the 3 guinea pigs. *Mycobacterium tuberculosis*, strain H37Rv, resistant to 0.2 γ of isoniazid per ml., was observed to produce visceral infection in one animal sacrificed at three weeks. However, the animals sacrificed at six weeks and nine weeks presented no gross lesions. The control strain H37Rv, susceptible to 0.06 γ of isoniazid per ml., produced progressive disease in all 3 animals, and all 3 animals had to be sacrificed at the end of the third week. It will be noted that some

[9] AMERICAN TRUDEAU SOCIETY: Am. Rev. Tuberc., 1946, *53*, 344.

TABLE 1

VIRULENCE IN GUINEA PIGS OF ISONIAZID-RESISTANT AND ISONIAZID-SUSCEPTIBLE TUBERCLE BACILLI

	NUMBER OF STRAINS USED	NUMBER OF ANIMALS INOCULATED PER STRAIN	ISONIAZID-SUSCEPTIBILITY OF ORGANISMS	WEEK ANIMAL SACRIFICED						TOTAL		ISONIAZID-SUSCEPTIBILITY OF RECOVERED ORGANISMS
				Progressive Disease			Number of Positive Cultures from Tissues			Number of Animals with Progressive Disease	Number of Cultures Isolated from Animal Tissue	
				3	6	9	3	6	9			
Army Medical Service Graduate School Experiment	11	3	Resistant to 50 γ per ml.	0	0	0	1	3	1	0	5	Resistant to 50 γ per ml.
	1	3	Resistant to 25 but not 50 γ	0	0	0	0	0	1	0	1	Resistant to 25 but not 50 γ
	1 (H37Rv)	3	Resistant to 0.2 γ per ml.	1	0	0	1	1	0	1	2	Resistant to 0.2 γ per ml.
	1 (H37Rv)	3	Susceptible to 0.06 γ per ml.	3	*		3			3	3	Susceptible to 0.06 γ per ml.
Fitzsimons Army Hospital, Research and Development Branch Experiment	15	1	Resistant to 100 γ per ml.	15†			14‡			15	14	Susceptible to 0.2 γ per ml.§

* All three animals inoculated with the susceptible strain lost weight and were sacrificed at three weeks.

† All animals were either dead or sacrificed at four weeks.

‡ Smear positive, but culture negative, for tubercle bacilli on one specimen.

§ One organism resistant to 5 γ of isoniazid per ml.

TABLE 2

VIRULENCE IN MICE OF ISONIAZID-RESISTANT AND ISONIAZID-SUSCEPTIBLE TUBERCLE BACILLI

NUMBER OF CF_1 MICE INOCULATED	MG. WEIGHT WET BACILLI USED PER ANIMAL AS INOCULUM*	ISONIAZID-SUSCEPTIBILITY IN γ PER ML.	GROSS LUNG INVOLVEMENT†
10	0.2	Susceptible to 0.06	4+
10	0.2	Resistant to 0.2	4+
10	0.2	Resistant to 25	3+
10	0.2	Resistant to 50	3+
10	0.5	Susceptible to 0.06	4+
10	0.5	Resistant to 0.2	4+
10	0.5	Resistant to 25	4+
10	0.5	Resistant to 50	4+

* Animals inoculated intravenously.

† Gross lung involvement:
4+ totally involved.
3+ 75 per cent involved.
2+ 50 per cent involved.
1+ 25 per cent involved.

strains were recovered from guinea pig tissues. The strains recovered were found to be isoniazid-resistant to the same drug concentration as when inoculated. Among the animals inoculated with strains resistant at the 25 and 50 γ concentration, enlarged spleens were found in several sacrificed at three weeks, but not in the animals sacrificed at six weeks or at nine weeks.

In the experiment conducted at Fitzsimons Army Hospital (table 1), all 15 animals inoculated with the fifteen so-called "resistant strains" developed progressive disease, and either died or were sacrificed at four weeks; all presented the characteristic picture of tuberculous infection. Bacilli isolated from these animals were, with one exception, susceptible to 0.2 γ of isoniazid per ml.

The evidence presented suggests that the cultures used in the Fitzsimons Army Hospital experiment were composed of mixed populations of isoniazid-susceptible and -resistant bacilli. This concept would perhaps explain the discrepancies in the literature concerning the virulence of isoniazid-resistant tubercle bacilli for the guinea pig.

In addition to guinea pig virulence studies, a limited number of studies were done at Walter Reed Army Medical Center on the virulence for mice of isoniazid-resistant and -susceptible tubercle bacilli. The H37Rv strain made resistant to 0.2, 25, and 50 γ of isoniazid per ml. was injected by way of a tail vein into 15-gm. white mice of the CF_1 strain (table 2). Each strain was inoculated into 10 mice. In the first study of this type, each mouse received 0.2 mg. of bacilli by wet weight. In a control series, 10 mice were inoculated with the H37Rv strain susceptible to isoniazid. The mice were sacrificed at twenty-eight days, and the gross lesions in the lung were evaluated according to the method of Raleigh and Youmans.[10] There was little difference in gross lesions observed in mice inoculated with susceptible or resistant tubercle bacilli. In a second study, mice were again inoculated by way of a tail vein; however, the inoculum was increased to 0.5

[10] RALEIGH, G. W., AND YOUMANS, G. P.: J. Infect. Dis., 1948, *82*, 205.

mg. of bacilli by wet weight, and the mice were sacrificed at twenty-one days. From gross inspection of the lungs of the animals, there was no difference noted in the amount of infection in the mice inoculated with resistant or susceptible bacilli.

In summary: These data indicate that tubercle bacilli resistant to 25 and 50 γ of isoniazid no longer cause progressive visceral disease in the guinea pig. There are also indications that strains resistant to as little as 0.2 γ of isoniazid per ml. are attenuated in virulence.

Mice infected with tubercle bacilli made resistant to 0.2, 25, and 50 γ of isoniazid per ml. show little difference in gross lesions from those inoculated with the susceptible parent strain.

The method of selection of isoniazid-resistant variants is important. The entire bacterial population must be resistant to the drug, or the loss of virulence for the guinea pig of isoniazid-resistant strains will be obscured.

It is suggested that variations in laboratory methodology may be the explanation for the discrepancies in the literature concerning the virulence in guinea pigs of isoniazid-resistant tubercle bacilli.

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THE ANTAGONISM OF THE TUBERCULOSTATIC ACTION OF ISONIAZID BY HEMIN

In another report by the present writer,[1] it was stated that hemin antagonized the tuberculostatic activity of isoniazid. It is the purpose of the present communication to describe in a preliminary form some details of this phenomenon. Additional and more detailed information regarding various factors involved in the hemin antagonism of isoniazid will be published at a later date.

Tests for hemin antagonism were based on changes in the minimal 100 per cent inhibitory concentrations of isoniazid, compared to the minimal 100 per cent inhibitory concentrations obtained in a basal synthetic medium.[1] Standardized suspensions of the H37Rv strain of *Mycobacterium tuberculosis* var. *hominis* were inoculated in twofold serial dilutions of isoniazid in medium with and without hemin, and the minimal 100 per cent inhibitory concentrations were determined by gross inspection after seven or fourteen days' incubation. In all cases, growth in the maximum tolerated isoniazid concentrations was subcultured and found to consist of isoniazid-susceptible cells.

As shown in table 1, hemin exhibited a competitive type of antagonism toward isoniazid. The maximum concentration of hemin which was employed, 320 γ per ml., effected about a twenty thousand fold reduction of isoniazid activity. This represents an astonishing degree of antagonism, far greater than that reported, for example, for certain keto compounds.[1, 2] Indeed, when tested under similar conditions, no biologic, organic, or inorganic material or compound of the many which have been examined here has exhibited such marked antagonistic activity for isoniazid inhibition. It should be mentioned that in this laboratory the following have also been found to act as isoniazid inactivators or antagonists: manganese salts, potassium cyanide, potassium ferricyanide, sodium azide, and sodium hydrosulfite. These, in addition to antagonists previously described,[1, 2, 3] namely, benzhydrazide, pyridoxal, pyruvate, and α-ketoglutarate, showed definite isoniazid antagonism but to a much less degree than that effected by hemin.

Additional studies have shown that the hemin antagonism of isoniazid occurred with other strains of mammalian tubercle bacilli, as well as with avian and saprophytic types of mycobacteria. Hemin, moreover, antagonized the tuberculostatic activity of other hydrazides, as salicylidene and benzylidene hydrazones, *p*-aminosalicylic acid hydrazide, α-picolinic acid hydrazide, and glycine hydrazide.

It was found that the addition of bovine serum or albumin to the basal medium almost completely nullified hemin-isoniazid antagonism. Because hemin readily forms a hemoprotein with albumin,[4] and because it was found here that various heme proteins, such as catalase, hemoglobin, and cytochrome C, did not antagonize isoniazid, it appears that only "free" hemin will act as an isoniazid antagonist.

A peculiar effect was obtained with manganese salts and hemin, one that may be termed "synergistic" antagonism. This is illustrated by the results given in table 2, where it can be seen that the antagonistic action of 1.0 γ of hemin per ml. was increased about one hundred and thirty fold by 10 γ of manganous chloride per ml., whereas individually each compound in the same concentrations exhibited a low degree of antagonism. In

[1] Fisher, M. W.: Am. Rev. Tuberc. (to be published).

[2] Schaefer, W. B.: Am. Rev. Tuberc., 1953, *68*, 273.

[3] King, M. B., *et al.*: Lancet, 1953, *2*, 573.

[4] Lemberg, R., and Legge, J. W.: Hematin Compounds and Bile Pigments, Interscience Publishers, New York, 1949.

TABLE 1

QUANTITATIVE ANTAGONISM OF ISONIAZID BY HEMIN

HEMIN, γ PER ML.	MAXIMUM ISONIAZID CONCENTRATION (γ PER ML.) SHOWING GROWTH*
320.0	125.0
160.0	62.5
80.0	31.3
40.0	15.6
20.0	12.5
10.0	6.25
8.0	6.25
6.0	3.13
4.0	1.56
2.0	0.39
1.0	0.098
0.8	0.049
0.6	0.049
0.4	0.024
0.2	0.012
0.0	0.006

* H37Rv, basal medium, 7 days' incubation

TABLE 2

EFFECT OF MANGANESE ON HEMIN ANTAGONISM OF ISONIAZID

$MnCl_2$ γ PER ML.	HEMIN γ PER ML.	M.I.C.* ISONIAZID γ PER ML.	— FOLD ANTAGONISM
0	0	0.012	—
10	0	0.024	2
0	1	0.195	16
10	1	25.0	2,048

* Minimal inhibitory concentration, H37Rv, basal medium, 7 days' incubation

similar experiments, little or no effect on hemin-isoniazid antagonism was obtained with salts of zinc, copper, iron, cobalt, calcium, and magnesium.

The possibility was considered that hemin acted in some manner outside the tubercle bacillus to inactivate isoniazid. However, biologic, chemical, and physico-chemical tests have failed to reveal that hemin causes extracellular or nonmetabolic isoniazid inactivation. This, taken into account with the apparently competitive nature of hemin-isoniazid antagonism, would suggest that isoniazid may act to interfere with the iron porphyrin metabolism of mycobacteria.

In summary: The tuberculostatic action of isoniazid was found to be markedly antagonized by hemin, apparently on a competitive basis. Such antagonism occurred only in a simple synthetic medium and was not apparent if serum or albumin were present. On the other hand, manganous ions appeared to act synergistically with hemin to increase antagonism. Present evidence indicates that isoniazid may inhibit the porphyrin metabolism of mycobacteria.

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October 5, 1953

ISONIAZID-RESISTANCE AND CATALASE ACTIVITY OF TUBERCLE BACILLI[1]

A Preliminary Report

It has long been known that the mycobacteria, like other aerobic bacteria, produce an iron-porphyrin-containing enzyme, *catalase*, decomposing hydrogen peroxide to molecular oxygen and water. Evidence is accumulating that isoniazid may react with iron-porphyrins under physiologic conditions.[2, 3] Therefore, grossly visible colonies of several strains of mycobacteria were submitted to the following test: Equal volumes of two solutions were freshly mixed, 30 per cent hydrogen peroxide (Superoxol, Merck) and 10 per cent Tween 80[4] in distilled water. Colonies on either solid oleic-acid–albumin type agar or inspissated egg-yolk-potato-glycerin (American Trudeau Society) culture media were covered with this solution at a temperature of approximately 20°C. The presence of the Tween 80 in this mixture was seen to permit better inundation of the hydrophobic colonies of tubercle bacilli on agar media, as well as to favor the persistence of any gas bubbles which might be formed. The following results have been observed:

Colonies of 3 parent, isoniazid-susceptible, laboratory strains of tubercle bacilli, H37Rv, Vallée, and Pearson, cultivated on an oleic-acid–albumin solid agar medium, as well as 10 freshly isolated, isoniazid-susceptible strains of human-type tubercle bacilli, were submitted to this test; they all gave positive reactions. A positive reaction consisted of the active evolution of grossly visible bubbles of gas from the submerged bacillary colonies within less than thirty seconds after application of the test solution described above.

Colonies of the isoniazid-resistant mutants of the same 3 laboratory strains of tubercle bacilli, which were selected and maintained by subculture in medium containing 50 γ of isoniazid per ml., gave negative reactions in this test. These particular mutants have been proved[5] to be highly attenuated or completely nonpathogenic for guinea pigs (in the case of the bovine strain, Vallée, markedly attenuated for both guinea pigs and rabbits). Microscopic examination of the colonies which had been submerged by the H_2O_2-Tween solution revealed that small gas bubbles were often promptly formed but that no further significant evolution of visible bubbles occurred even on prolonged observation.

The colonies of 15 different isoniazid-resistant strains of tubercle bacilli (resistant to more than 10 γ of isoniazid per ml.) on primary isolation from sputum on the American Trudeau Society medium or on an oleic-acid–albumin solid agar medium, from as many patients treated with isoniazid, were submitted to this test; they gave negative reactions.

Colonies of 2 isoniazid-resistant strains of tubercle bacilli, cultivated on a modification of the oleic-acid–albumin solid agar medium, gave moderate but definitely positive reactions in this test. These 2 strains proved to consist predominantly of organisms resistant to 10 γ of isoniazid per ml. but susceptible to 25 γ of isoniazid per ml. and to be capable of causing acute, fatal infection in guinea pigs when introduced intravenously in large doses. These strains were incapable of producing progressive visceral tuberculosis in guinea pigs when the animals were challenged subcutaneously in the groin with large inocula.

[1] This study was aided by a grant from the Committee on Medical Research of the American Trudeau Society, medical section of the National Tuberculosis Association.

[2] Fisher, M.: Personal communication, May, 1953.

[3] Gray, C. T.: Bacteriol. Proc. Soc. Am. Bacteriologists, 1953, p. 89.

[4] Tween 80 is the proprietary name for the polyoxyethylene derivative of Sorbitan monooleate, obtainable from the Atlas Powder Co., Wilmington, Delaware.

[5] Middlebrook, G., and Cohn, M. L.: Science, 1953, *118*, 297.

Colonies of several attenuated or avirulent isoniazid-susceptible strains of tubercle bacilli (a BCG strain, the H37Ra variant of the H37Rv strain, and a laboratory strain designated CMHA) all gave positive reactions in this test.

Two saprophytic, nonpathogenic strains, *M. phlei* and *M. smegmatis*, natively resistant to much higher concentrations of isoniazid *in vitro* than are native strains of mammalian tubercle bacilli, gave much stronger reactions in this test than did the isoniazid-susceptible strains of tubercle bacilli.

Old, spontaneously autolyzed, cultures of isoniazid-susceptible strains of tubercle bacilli on the American Trudeau Society medium often gave negative or doubtful reactions.

Cultures of 3 strains of tubercle bacilli which proved, on being plated out on an oleic-acid–albumin solid agar medium containing various concentrations of isoniazid, to consist of mixed populations of isoniazid-susceptible and isoniazid-resistant organisms gave weakly to moderately positive reactions in this test.

The failure of colonies of isoniazid-resistant mutants to give a positive reaction in this test was not influenced by the omission of isoniazid from the culture medium on which the tested colonies were grown, provided such mutant strains were not repeatedly subcultured in the absence of isoniazid.

In summary: Mutants of several strains of virulent tubercle bacilli which are highly resistant to the antimicrobial activity of isoniazid *in vitro*, and whose pathogenic properties are markedly attenuated for guinea pigs under certain experimental conditions, manifest no "catalase activity" *in vitro* either in the presence, or in the absence, of isoniazid.

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FILTERABLE FORMS OF MYCOBACTERIUM TUBERCULOSIS

To the Editor of the American Review of Tuberculosis:

In a recent letter to this journal (Nègre, L., and Bretey, J.: Am. Rev. Tuberc., 1953, *68*, 467), Nègre and Bretey have endeavored to "separate the erroneous interpretation from the facts" in the thirty-year-old controversy about filterable forms of *Mycobacterium tuberculosis*.

It may be useful also to examine this question briefly from the morphologic viewpoint. During the past ten years, as we have shown in a recent review (Werner, G. H.: Advances in Tuberculosis Research, 1951, *4*, 53), the cytologic study of *M. tuberculosis* has been advanced considerably through the use of electron-microscopic methods. A cell wall, a cytoplasmic membrane, vacuoles, cytoplasmic granules assimilated to mitochrondria (Winterscheid, L. C., and Mudd, S.: Am. Rev. Tuberc., 1953, *67*, 59), and a nuclear apparatus similar to the one observed in other bacteria have been recognized. Morphologic differences with the method of cultivation, the age of the culture, the strain employed, and the virulence of the strain (Werner, G. H.: Presse méd., 1951, *50*, 1043) have also been described. In no case, however, was it possible, with the electron microscope, to find evidence for the presence of stages resembling the "filterable forms" previously described with light-microscopic methods.

Nocard and Roux (Nocard, E., and Roux, E.: Ann. Inst. Pasteur, 1887, *1*, 24) and Metchnikoff (Metchnikoff, E.: Virchows Arch. f. path. Anat., 1888, *113*, 63) knew long ago that, besides binary fission, other modes of division, such as budding or branching, exist in *M. tuberculosis*. This has been confirmed by electron microscopy, but these modes of division do not produce daughter-cells which differ in size or in shape from the ordinary bacilli. On the other hand, it is possible by various methods to fragment the cytoplasm of *M. tuberculosis* into small, spherical units which possess the same antigenic properties as killed tubercle bacilli (Laporte, R.: Ann. Inst. Pasteur, 1943, *69*, 262). These particles can also be seen under the electron microscope, but they do not multiply in artificial media nor do they cause progressive disease in susceptible animals.

Although electron-microscopic studies have hitherto failed to bring forth any unquestionable evidence for the existence of true filterable forms in *M. tuberculosis*, some observations may partly explain the results of filtration experiments, and this explanation seems to agree with the one offered by Nègre and Bretey (*see* above).

By growing tubercle bacilli on a thin collodion membrane covering a plate of oleic-acid-albumin agar, it is possible to study them under the electron microscope with a minimum disturbance of their natural appearance. After several days of incubation, a small piece of the collodion membrane is transferred onto an electron-microscopic screen and examined directly. Only relatively young cultures can be studied by this method, since the growth soon becomes abundant, leading to specimens too opaque for electron microscopy.

With this technique, examining the pictures obtained of a ten-day-old culture of the H37Rv human strain, for instance, one is struck by several facts. First, the marked *flexibility* of the cell wall of the tubercle bacillus is evident in the "cords" where the bacilli are pressed against one another. Instead of straight, rigid rods, one observes bacilli which are bent in all directions, distorted, and stretched. In some bacilli, the elastic cell wall seems to be moulded to the surface of its immediate neighbors; this surface often shows small, pseudopod-like protrusions which fit closely into corresponding depressions in the surface of adjacent bacilli.

The extreme *ductility* of the cell wall of *M. tuberculosis* is coincidentally demonstrated when, under a strong electron beam, the supporting collodion membrane breaks and rolls up. Any bacillus which happens to lie on that membrane on both sides of the cut is then submitted to a considerable traction, but it will usually stretch up to twice its original length before breaking. Similar observations, on avian tubercle bacilli, have led Knaysi and associates (Knaysi, G., Hillier, J., and Fabricant, C.: J. Bact., 1950, *60*, 423) to state that, in those bacilli "the cell wall is ductile to an unusual degree."

Among the numerous factors involved in the ability of microorganisms to pass through bacteriologic filters, there can be no doubt that the ductility and elasticity of the cell wall, as well as the deformability of the entire cell, play an important role. This is especially true for nonmotile species, such as *M. tuberculosis*. Bacteria endowed with a highly ductile and elastic cell wall will find their way more easily through the staggered pores of porcelain filters, especially if the applied pressure is increased above the normal value.

Ductility and elasticity of the cell wall vary to a great extent among the bacterial species and also within the same species, with varying conditions. For instance, electron-microscopic studies of *M. tuberculosis* have shown that, in older cultures in liquid media, the bacilli are shorter and even coccoid in shape and that they lose that marked ductility, their cell wall becoming much more rigid, less deformable. This observation may explain why, as pointed out by Nègre and Bretey, the passage of viable bacterial elements through bacteriologic filters has been noted only when using *young cultures* of *M. tuberculosis*.

Nègre and Bretey also suggest that the smaller lipid content of young bacilli may be a reason for their special behavior. Indeed, it has been shown by Asselineau (Asselineau, J.: Ann. Inst. Pasteur, 1951, *81*, 306) that some lipids are present in smaller quantities in bacilli from young cultures. In addition, electron microscopy of older tubercle bacilli previously extracted with hexane indicates the great extent to which the lipids account for their cytoplasmic mass (Werner, G. H.: Advances in Tuberculosis Research, 1951, *4*, 79). One may assume then that young bacilli, containing a smaller amount of *cytoplasmic* lipids, will have less difficulty than older ones in passing through the holes of filters.

Furthermore, electron microscopy has revealed that, in cultures of the human virulent strain H37Rv, two types of bacilli can be recognized (*see* Werner, above): those in which the cytoplasm is relatively opaque to the electron beam and those in which the cytoplasm is extremely transparent. The ductility of the cell wall is even more evident in the transparent bacilli, and the lesser opacity of their cytoplasm to the electrons suggests that some material, present in the more opaque bacilli, is either absent altogether in this instance or present in smaller amount. If these transparent bacilli are the ones which pass more easily through bacteriologic filters, one might then expect that the biologic properties of the filtrate would differ somewhat from those of the original culture.

In summary, among the factors involved in the filterability of bacilli from young cultures of *M. tuberculosis*, one should, in our opinion, consider the marked ductility and elasticity of their cell wall as well as their lesser content of cytoplasmic lipids, features which may be more pronounced in some bacilli than in others within the same culture.

Respectfully yours,

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November 1, 1953

Books

MÉNINGITE TUBERCULEUSE AND TUBERCULOSE MILIAIRE DE L'ENFANT: LEUR TRAITEMENT. By Robert Debré and H.-E Brissaud. Pp. 632, Masson and Cie, Paris, 1953.

The authors report in this book their experience with antimicrobial therapy in 644 cases of tuberculous meningitis in children observed during a period of five years. From this vast experience they have written a treatise on the disease which covers all the new aspects with which the physician has to be familiar in order to obtain the maximum benefit from the therapeutic means actually at his disposal. They begin this treatise by defining the epidemiologic conditions under which the disease is most likely to occur. They describe the symptomatology of the prodromal stage and of the onset of the disease and outline the elements on which an early diagnosis can be based. They follow the disease in its variety of evolutions, relapses, and complications. They show how these different eventualities can be recognized, and often predicted before the appearance of clinical signs, from the cytologic, chemical, and bacteriologic findings in the cerebrospinal fluid, from the results of the opthalmologic examination, and from the encephalogram. All of these methods of investigation are analyzed in their many details.

Special attention is paid to the problems of evaluation of therapy, since the same treatment may give very different results depending upon whether it is applied at an early or at a more advanced stage of the disease. The elements are outlined upon which the prognostic evaluation of each case can be based before treatment.

In the chapter on treatment, the authors consider the various forms of drug therapy, the problems of dosage and duration of chemotherapy, and the indications for neurosurgical interventions. They advocate a generally and locally applied polyvalent drug therapy of long duration, with streptomycin in moderate doses, which has permitted nearly complete elimination of neurotoxic sequelae. In a last chapter the authors summarize their experiences with 170 cases of miliary tuberculosis.

This remarkable book is written in an elegant style. It is documented by numerous case reports, statistics, illustrations of the pathologico-anatomical findings, and bibliographic references. Its importance can hardly be overemphasized.

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EFFECTIVE INHALATION THERAPY. By Edwin Rayner Levine, Pp. 157, National Cylinder Gas Company, Medical Division, Chicago, Illinois.

In this 157-page book the author attempts to illustrate the mechanism by which oxygen lack is produced in clinical illness in the hope that such graphic illustrations will make clear the rationale of the use of inhalation therapy. The author is aided in this effort by 49 superb cartoon-like diagrams in color. Dr. Levine reviews the physiology of respiration, the gas exchange between the alveoli and the blood stream, and the particular problems in such situations as pneumonia, bronchial obstruction, pulmonary edema, and emphysema. Emphasis is placed on the importance of careful observation and the use initially of low concentrations of oxygen in the treatment of patients with chronic oxygen lack in order to avoid respiratory depression and CO_2 narcosis. In the discussion of the treatment of pulmonary edema, emphasis is also given to the importance of starting with no positive pressure when the mask is first applied to avoid producing a sense of oppression and

labored breathing, then gradually increasing to 4 cm. of water-positive pressure, and finally reducing the positive pressure gradually in order to avoid the return of pulmonary edema.

Dr. Levine and the publishers have directed this effort principally at nurses and technical personnel and have provided a glossary of medical terms. The text lacks, however, the clear scientific exposition with reference to original investigation as well as the bibliography which make other treatises on inhalation therapy more valuable for the physician.

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THE CONCEPTION OF DISEASE. Its History, Its Versions, and Its Nature. By Walter Riese. Pp. 120, Philosophical Library, New York, Price, $3.75.

This monograph is a study of the various concepts of disease held in the history of man. The concepts discussed include Stoic, Platonic, anthropologic, moral, Hippocratic, Galenic, anatomic, etiologic, social, psychologic, ontologic, biographic, and metaphysic. The author points out in the foreword that all of these various concepts exist side by side at each stage of human history.

All elements are considered to be always present, with the emphasis being changed from time to time, depending upon the prominent teachers of the day and the prevailing scientific, historic, social, and philosophic influences.

There is doubtless much of interest to the medical historian and the philosopher in this book. The nature of the topic and its scope make it a difficult subject to treat. The various concepts of disease have been somewhat artificially separated and lifted out of context in order that they may be discussed individually. In addition, it is apparent that much of the categorization depends upon the author's own opinions. This may be necessary to achieve the purpose of the monograph but the over-all effect is of poor integration.

LOUISE H. ORMOND
Seattle, Washington

BOOKS RECEIVED

DIE BEHANDLUNG DER KNOCHEN- UND GELENKTUBEKULOSE. By Heinz May. Pp. 221, with illustrations, Ferdinand Enke Verlag, Stuttgart, 1953.

FUNDAMENTALS OF RADIOGRAPHY. Eighth Edition. Pp. 100, Medical Division, Eastman Kodak Company, Rochester, New York, 1953.

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American Trudeau Society

Medical Section of the National Tuberculosis Association

NOTES

INTERNATIONAL UNION AGAINST TUBERCULOSIS

The International Union Against Tuberculosis will hold its Thirteenth Session in Madrid, Spain, September 26–October 2, 1954, under the patronage of General Francisco Franco. Presiding over the conference will be Professor Crespo Alvarez, president of the Union.

Meetings of the Conference of Executive Directors of National Tuberculosis Associations, the Executive Committee, and the Union's governing body, the Council, will be held September 24 and 25.

Scientific sessions on the social, biological, and clinical aspects of tuberculosis have been planned, with the following topics and main speakers:

"Bacteriological and Anatomical Changes in Tuberculous Lesions Under Antibiotic and Chemotherapeutic Treatment"—Dr. George Canetti, Pasteur Institute, Paris; "Indications and Choice of Surgical Procedures in Patients with Pulmonary Tuberculosis Treated by Chemotherapy"—Dr. Kjeld Torning, Øresundshospital, Copenhagen, and Dr. Clarence Crafoord, Söderby Hospital for Tuberculosis, Stockholm; and "Changes in the Control of Tuberculosis as a Result of Modern Therapy"—Dr. Blanco Rodriguez, Madrid.

The program will also include a special symposium on animal tuberculosis, planned in connection with a veterinary congress which is being held in Madrid at the time of the Union session.

THIRD INTERNATIONAL CONGRESS ON DISEASES OF THE CHEST

The Third International Congress on Diseases of the Chest will be held October 4–8, 1954, in Barcelona, Spain.

Free communications will be held on Diseases of the Chest and Thoracic Surgery: Anesthesiology, Experimentation, Tuberculosis, Cancer, Thoracic Wall, Lungs, Pleura, Mediastinum, Esophagus, Pericardium, Heart, Great Vessels, and Diaphragm.

The Congress will meet in Sections corresponding to each of the thoracic specialities. At the end of the meetings there will be an hour of discussion to be held on the themes, which will be announced in time.

Five minutes will be given as a minimum and fifteen as a maximum for the lecture of the summaries.

During the celebration of the Congress an exposition of surgical, clinical, radiological, and photographic materials as well as pharmaceutical products, books, and arts related to medicine will be installed at the "Hospital de la Santa Cruz y de San Pablo" of Barcelona.

The official languages will be: Spanish, English, and French.

All physicians and surgeons who wish to attend this Congress may write for the program as well as for any other information to the General Secretary of the Congress: Córcega, 393, 4°. 1ª.—Barcelona, Spain.

DR. SCHEPERS NAMED DIRECTOR OF THE SARANAC LABORATORY

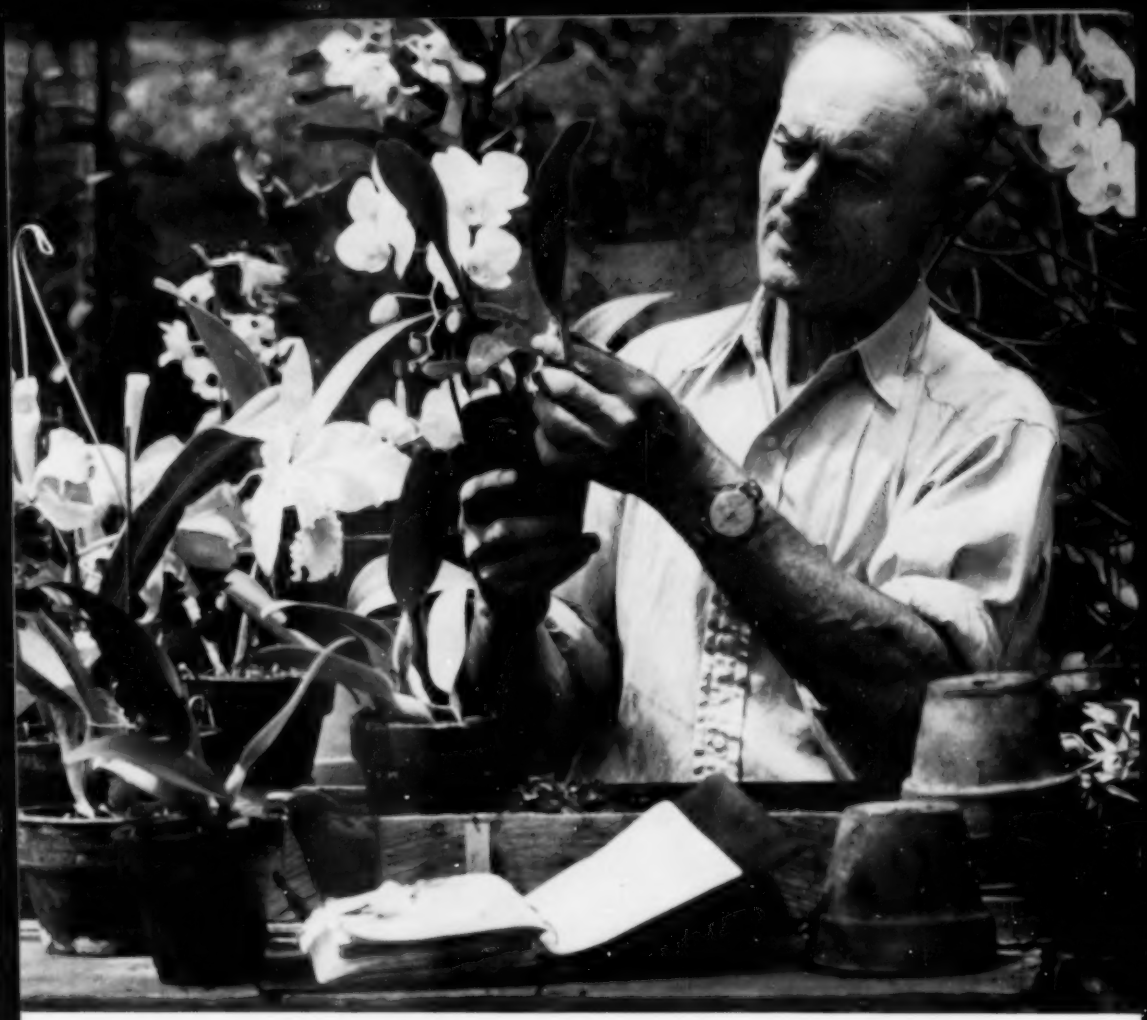
Dr. Gerrit Willem Hendrik Schepers of Johannesburg, South Africa, has been named Director of the Saranac Laboratory of the Trudeau-Saranac Institute, Trudeau, New York. He is expected to assume his new duties in the late spring.

Under Dr. Schepers' direction, it is expected that the work of the Saranac Laboratory conducted by previous directors, Drs. Edward R. Baldwin, Leroy U. Gardner, and Arthur J. Vorwald, will be continued and expanded.

ANNUAL MEETING OF THE AMERICAN SOCIETY FOR CLINICAL INVESTIGATION

The forty-sixth annual meeting will be held in Atlantic City, New Jersey, on Monday, May 3, 1954, at 9:00 a.m. Business meetings will precede the morning and afternoon scientific sessions. Headquarters will be at the Chalfonte-Haddon Hall Hotel. The meetings will be held in the Steel Pier Theater.

Reservations at Chalfonte-Haddon Hall may be made at the following rates: double room, each person, $4.50 to $11.00; single occupancy, $6.00 to $10.00.



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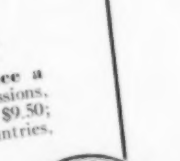

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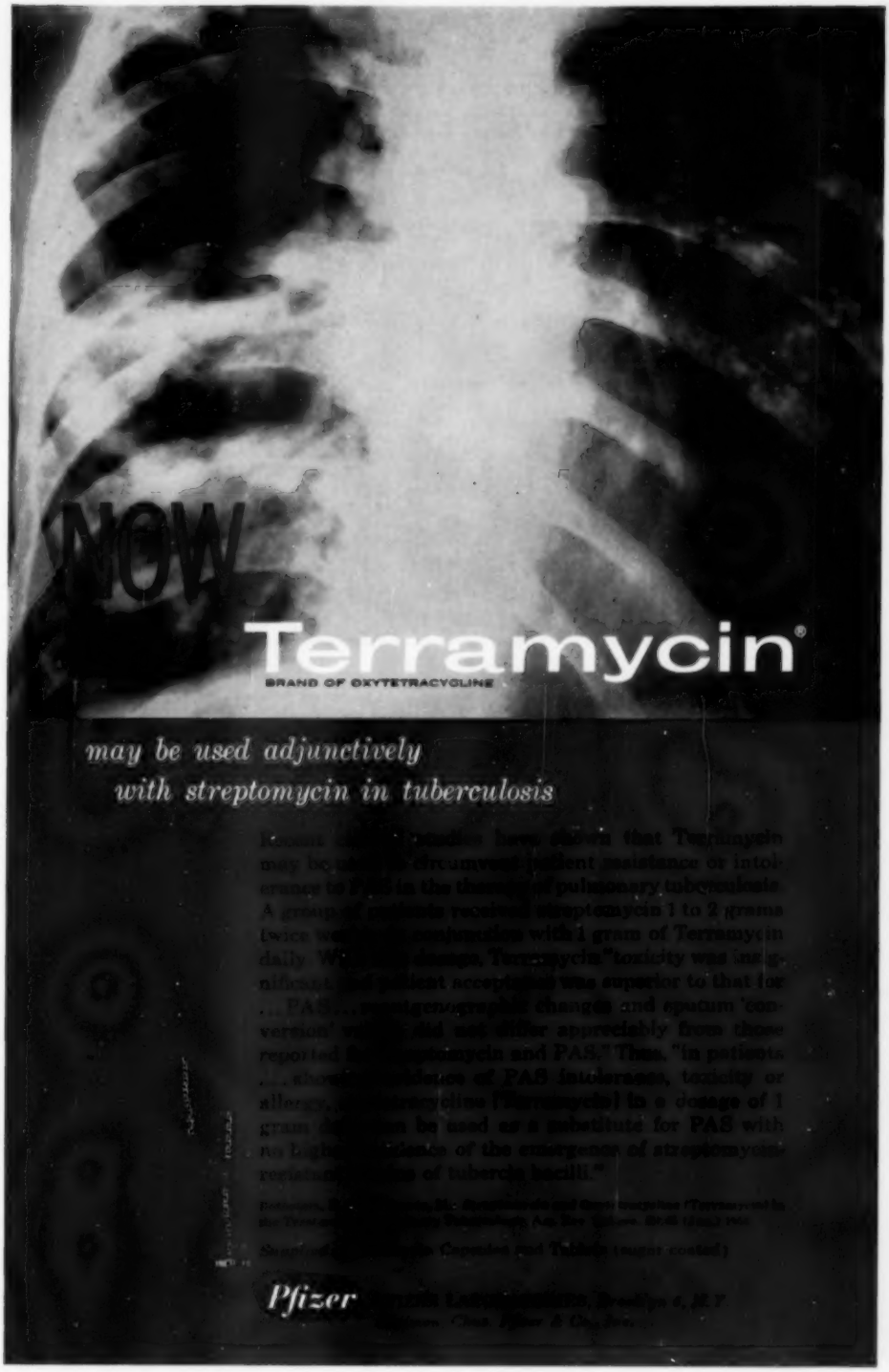
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50th ANNIVERSARY ★ NATIONAL TUBERCULOSIS ASSOCIATION
Annual Meeting, Atlantic City, New Jersey, May 17-21, 1954

The 50th Anniversary of the National Tuberculosis Association will be celebrated at its Annual Meeting to be held in Atlantic City, New Jersey, May 17-21, 1954.

Three adjacent hotels, the Ambassador, Chelsea and Ritz-Carlton, will serve as headquarters. Sessions and conferences will be divided among the three hotels with no one hotel marked exclusively for medical or community service programs.

Applications for rooms should be made directly to the hotel of choice. In the event that the hotel is unable to provide the desired space, requests will be turned over to the Convention Bureau, which will clear requests with another one of the cooperating hotels.

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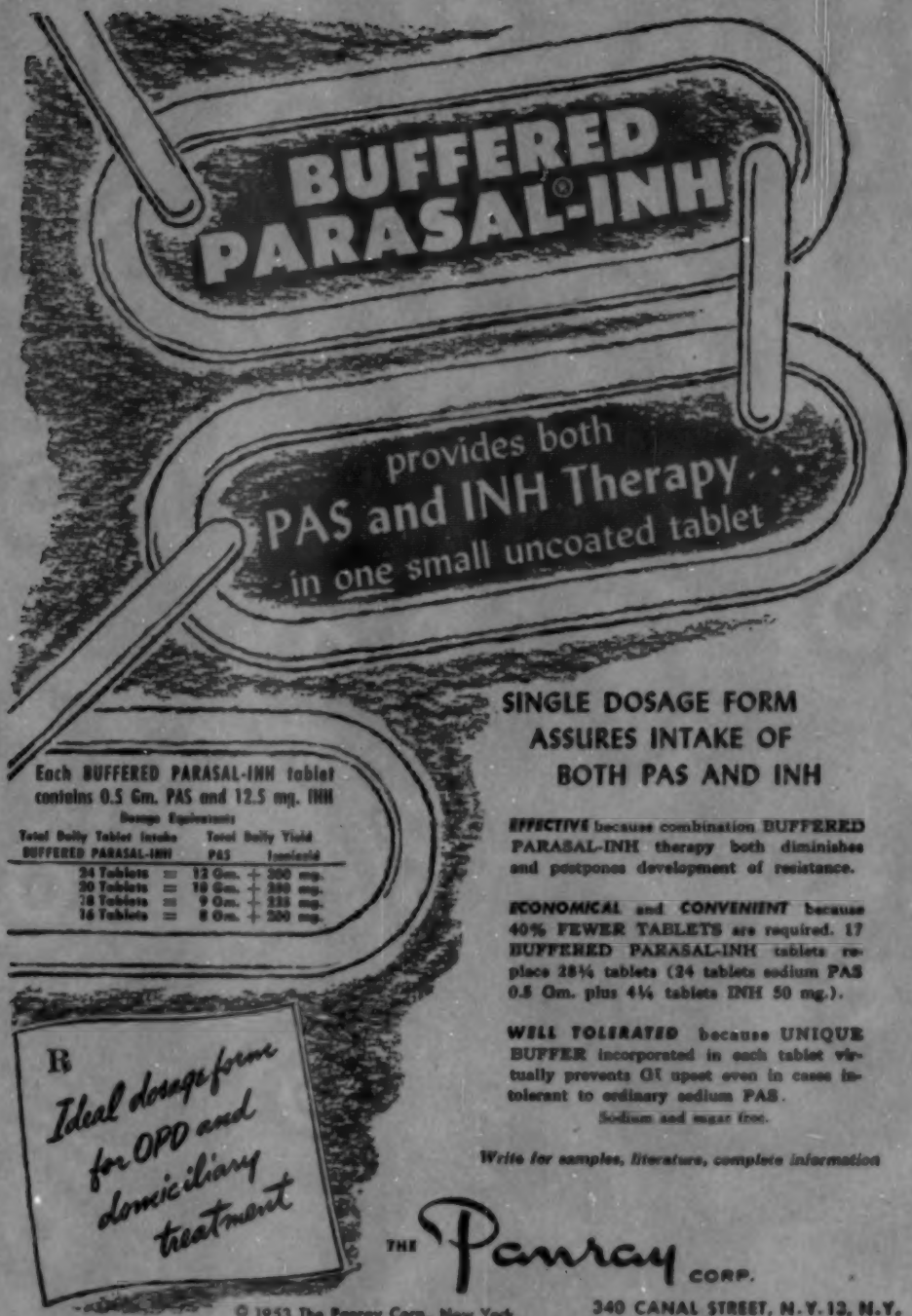

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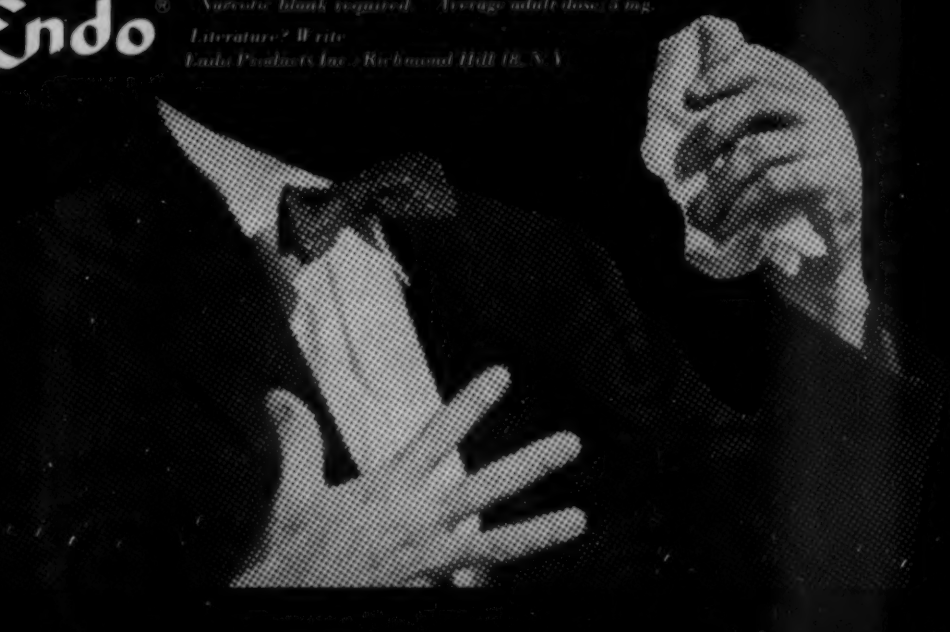
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